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NORINYL® 1+50

tablets (norethindrone 1 mg with mestranol 0.05 mg)

Special Note

Oral contraceptives have been marketed in the United States since 1960. Reported pregnancy rates vary from product to product. The effectiveness of the sequential products appears to be somewhat lower than that of the combination products. Both types provide almost completely effective contraception.

An increased risk of thromboembolic disease associated with the use of hormonal contraceptives has now been shown in studies conducted in both Great Britain and the United States. Other risks, such as those of elevated blood pressure, liver disease and reduced tolerance to carbohydrates, have not been quantitated with precision.

Long term administration of both natural and synthetic estrogens in subprimate animal species in multiples of the human dose increases the frequency for some animal carcinomas. These data cannot be transposed directly to man. The possible carcinogenicity due to the estrogens can neither be affirmed nor refuted at this time. Close clinical surveillance of all women taking oral contraceptives must be continued.

Indications

NORINYL 1+50 21-DAY is indicated for oral contraception.

Contraindications

1. Thrombophlebitis, thromboembolic disorders, cerebral apoplexy, or a past history of these conditions.
2. Markedly impaired liver function.
3. Known or suspected carcinoma of the breast.
4. Known or suspected estrogen dependent neoplasia.
5. Undiagnosed abnormal genital bleeding.
6. Known or suspected pregnancy.

Warnings

1. The physician should be alert to the earliest manifestations of thrombotic disorders (thrombophlebitis, cerebrovascular disorders, pulmonary embolism, and retinal thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. Retrospective studies of morbidity and mortality in Great Britain and studies of morbidity in the United States have shown a statistically significant association between thrombophlebitis, pulmonary embolism, and cerebral thrombosis and embolism and use of oral contraceptives. There have been three principal studies in Britain[1,2,3] leading to this conclusion, and one[4] in this country. The estimate of the relative risk of thromboembolism in the study by Vessey and Doll[3] was about sevenfold, while Sartwell and associates in the United States found a relative risk of 4.4, meaning that the users are several times as likely to undergo thromboembolic disease without evident cause as non-users. The American study also indicated that the risk did not persist after discontinuation of administration, and that it was not enhanced by long continued administration. The American study was not designed to evaluate a difference between products. However, the study suggested that there might be an increased risk of thromboembolic disease in users of sequential products. This risk cannot be quantitated, and further studies to confirm this finding are desirable.
2. Discontinue medication pending examination if there is sudden partial or complete loss of vision, or if there is a sudden onset of proptosis, diplopia or migraine. If examination reveals papilledema or retinal vascular lesions, medication should be withdrawn.
3. Since the safety of NORINYL 1+50 21-DAY in pregnancy has not been demonstrated, it is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule the possibility of pregnancy should be considered at the time of the first missed period.
4. A small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The long range effect to the nursing infant cannot be determined at this time.
5. Hepatic cell adenomas have been reported in women on oral contraceptives. The adenoma may present as an abdominal mass or with the signs and symptoms of an acute abdomen. A bleeding hepatic cell adenoma should be considered if the patient has abdominal pain or evidence of intra-abdominal bleeding.



The norethindrone/mestranol 1+50 combination. From the company that developed it.

Precautions

1. The pretreatment and periodic physical examinations should include special reference to liver, breasts and pelvic organs, including Papanicolaou smear since estrogens have been known to produce tumors, some of them malignant, in five species of subprimate animals.
2. Endocrine and possibly liver function tests may be affected by treatment with NORINYL 1+50 21-DAY. Therefore, if such tests are abnormal in a patient taking NORINYL 1+50 21-DAY, it is recommended that they be repeated after the drug has been withdrawn for 2 months.
3. Under the influence of estrogen-progestogen preparations, pre-existing uterine fibromyomata may increase in size.
4. Because these agents may cause some degree of fluid retention, conditions which might be influenced by this factor, such as epilepsy, migraine, asthma, cardiac or renal dysfunction, require careful observation.
5. In breakthrough bleeding, and in all cases of irregular bleeding per vaginam, nonfunctional causes should be borne in mind. In undiagnosed bleeding per vaginam, adequate diagnostic measures are indicated.
6. Patients with a history of psychic depression should be carefully observed and the drug discontinued if the depression recurs to a serious degree.
7. Any possible influence of prolonged NORINYL 1+50 21-DAY therapy on pituitary, ovarian, adrenal, hepatic or uterine function awaits further study.
8. A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. The mechanism of this decrease is obscure. For this reason, diabetic patients should be carefully observed while receiving NORINYL 1+50 21-DAY therapy.
9. The age of the patient constitutes no absolute limiting factor, although treatment with NORINYL 1+50 21-DAY may mask the onset of the climacteric.
10. The pathologist should be advised of NORINYL 1+50 21-DAY therapy when relevant specimens are submitted.
11. Susceptible women may experience an increase in blood pressure following administration of contraceptive steroids.

Adverse Reactions Observed in Patients Receiving Oral Contraceptives

A statistically significant association has been demonstrated between use of oral contraceptives and the following serious adverse reactions: thrombophlebitis, pulmonary embolism, cerebral thrombosis.

Although available evidence is suggestive of an association, such a relationship has been neither confirmed nor refuted for the following serious adverse reactions: neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions are known to occur in patients receiving oral contraceptives: nausea, vomiting, gastrointestinal symptoms (such as abdominal cramps and bloating), breakthrough bleeding, spotting, change in menstrual flow, amenorrhea during and after treatment, edema, chloasma or melasma, breast changes: tenderness, enlargement and secretion, change in weight (increase or decrease), changes in cervical erosion and cervical secretions, suppression of lactation when given immediately post-partum, cholestatic jaundice, migraine, rash (allergic), rise in blood pressure in susceptible individuals, mental depression.

Although the following adverse reactions have been reported in users of oral contraceptives, an association has been neither confirmed nor refuted: anovulation post treatment, premenstrual-like syndrome, changes in libido, changes in appetite, cystitis-like syndrome, hirsutism, loss of scalp hair, erythema multiforme, headache, nervousness, dizziness, fatigue, backache, erythema nodosum, hemorrhagic eruption, itching.

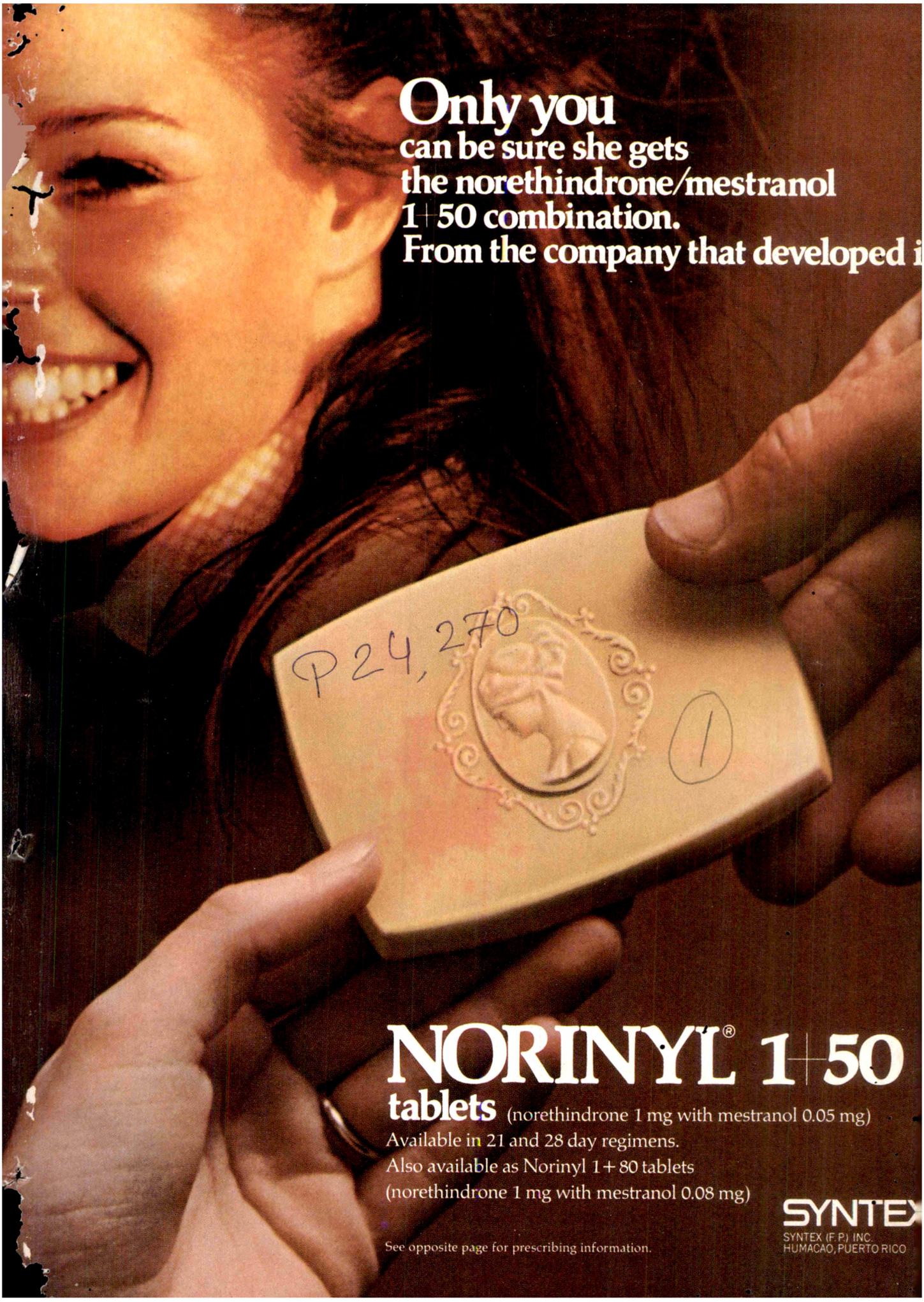
The following laboratory results may be altered by the use of oral contraceptives: hepatic function: increased sulfobromophthalein retention and other tests, coagulation tests: increase in prothrombin factors VII, VIII, IX, and X, thyroid function: increase in PBI, and butanol extractable protein bound iodine and decrease in T^3 uptake values, metyrapone test, pregnanediol determination.

[1]Royal College of General Practitioners: Oral Contraception and Thromboembolic Disease. J. Coll. Gen. Pract., 13:267-279, 1967.
[2]Inman, W.H.W. and Vessey, M.P. Investigation of Deaths from Pulmonary Coronary and Cerebral Thrombosis and Embolism in Women of Child-Bearing Age, Brit Med J, 2:193-199, 1968.
[3]Vessey, M.P. and Doll, R. Investigation of Relation between Use of Oral Contraceptives and Thromboembolic Disease. A Further Report. Brit Med J, 2:651-657, 1969.
[4]Sartwell, P.E., Masi, A.T., Arthes, F.G., Greene, G.R. and Smith, H.E., Thromboembolism and Oral Contraceptives: An Epidemiological Case-Control Study. Am. J.Epidem. 90:365-380, (Nov.) 1969.

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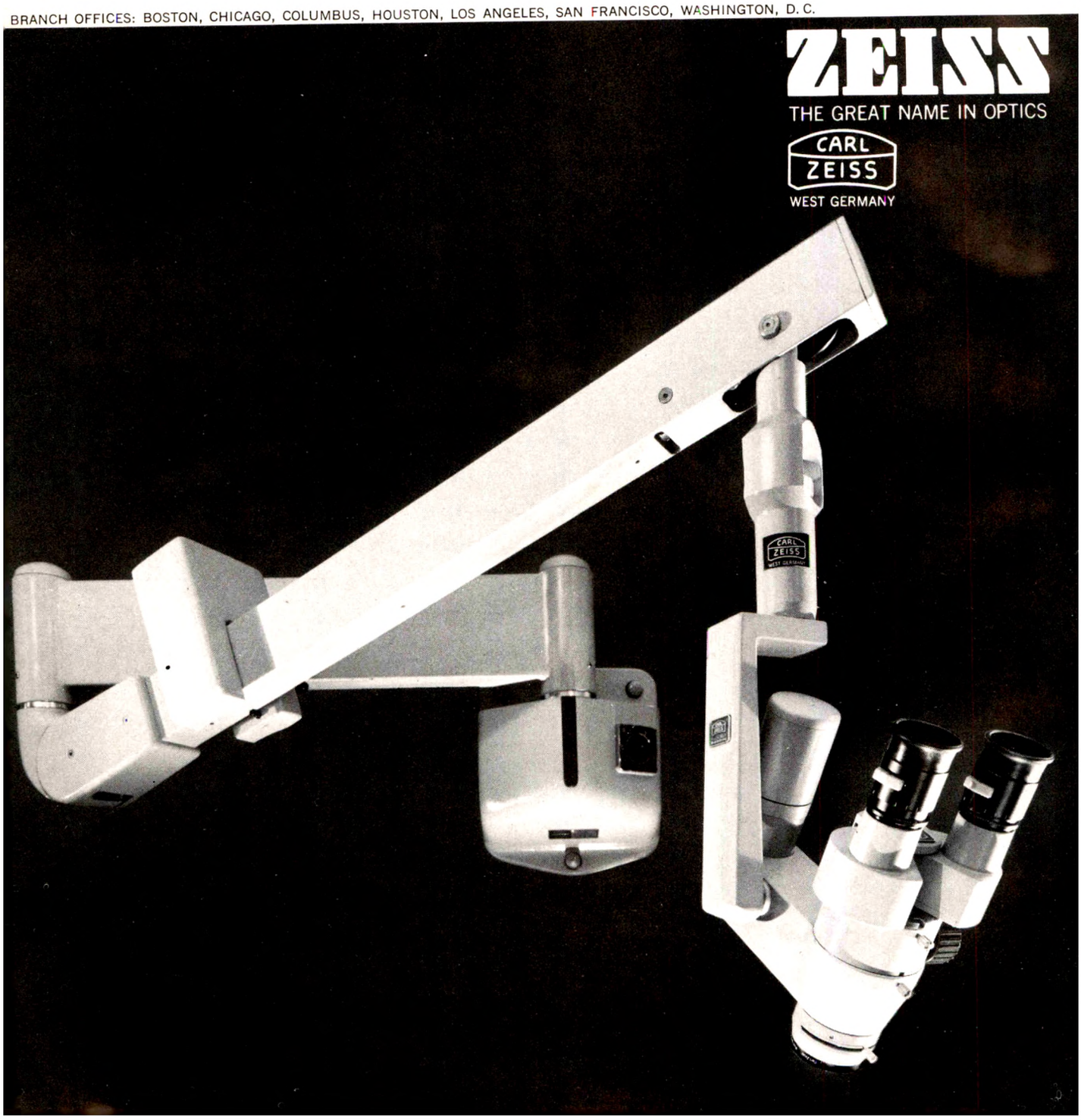
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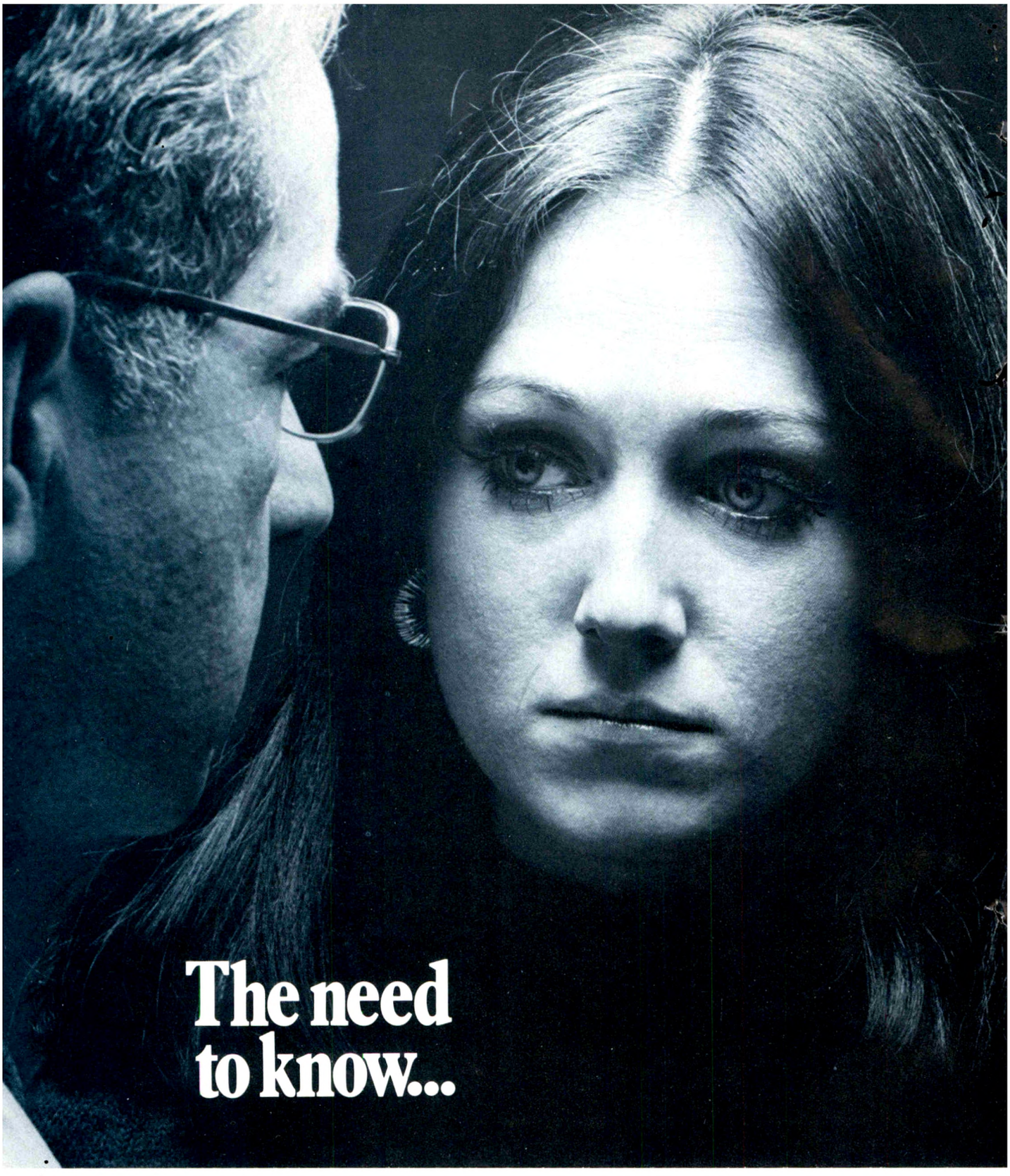
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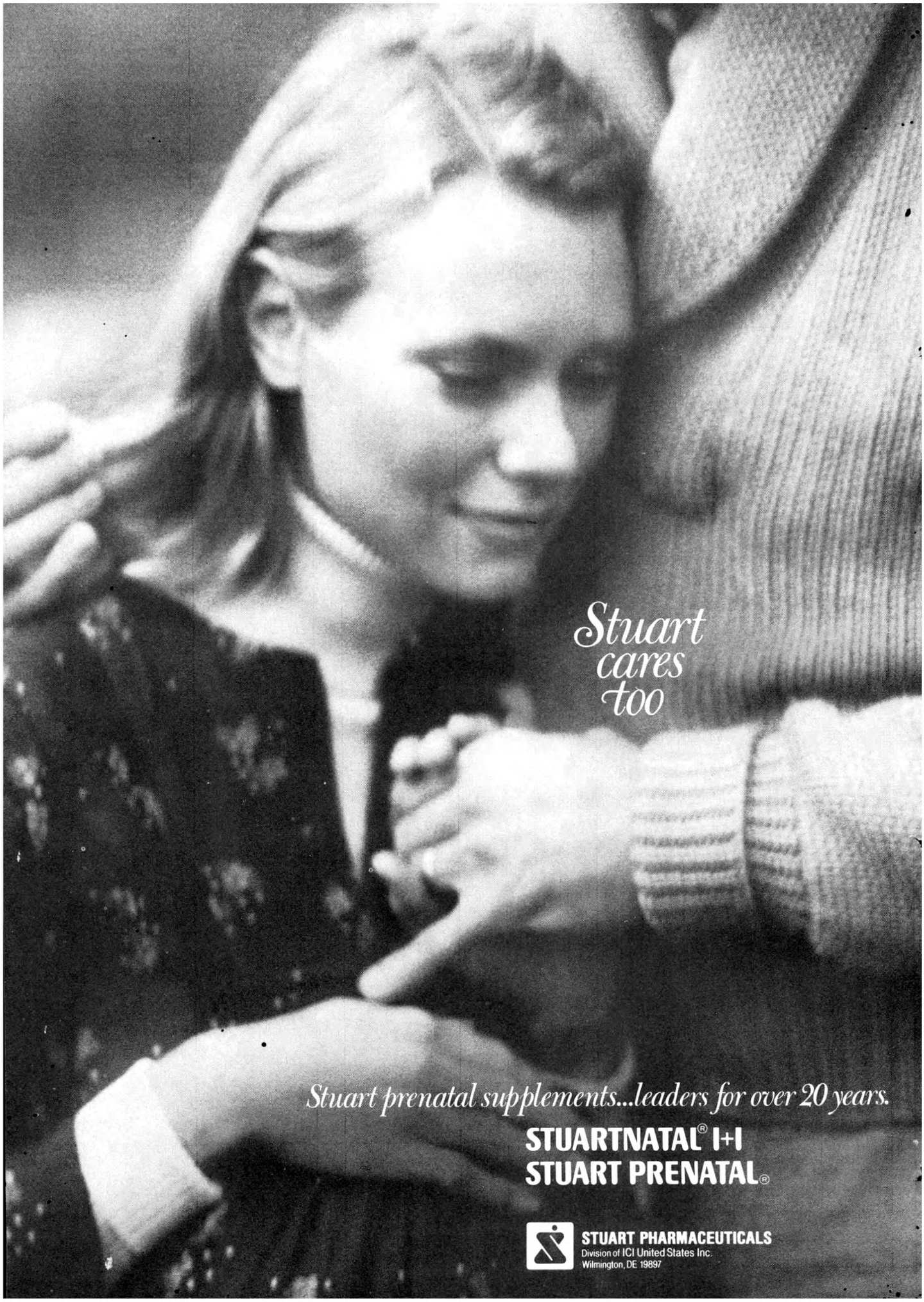
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All articles published in this Journal must be contributed to it exclusively. Articles previously published in another language are not acceptable.

It is assumed by the Editors that articles emanating from a particular institution are submitted with the approval of the requisite authority, including all matters pertaining to human studies.

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Manuscripts. Manuscripts should be typewritten on one side of the paper only, with double spacing and liberal margins. The original and one copy of the manuscript are required. References should be placed at the end of the article and should conform to the style of the Cumulated Index Medicus, viz., name of author, article title, name of periodical, volume, page, and year. Authors are encouraged to limit references to *16, except* for Communications in Brief, limited to *2,* Current Investigation and Clinical Opinion, limited to *6,* and Current Developments for which there is *no* limit. Illustrations accompanying manuscripts should be numbered, provided with suitable legends, and marked lightly on the back with the author's name. Authors should indicate on the manuscript the approximate position of tables and text figures.

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An abstract of 50 to 150 words, to be published as an introduction, should accompany each manuscript.

A footnote should be included which gives the name and complete mailing address of the person to whom reprint requests and correspondence should be sent.

A Guide to Writing for the American Journal of Obstetrics and Gynecology, giving further details of how a manuscript should be prepared, may be obtained from the Publisher on request.

Illustrations. A reasonable number of halftone illustrations will be reproduced free of cost to the author, but special arrangements must be made with the Editors for color plates, elaborate tables, or extra illustrations. Original drawings or graphs should be drawn with black India ink. Typewritten or freehand lettering is not acceptable. All lettering must be done professionally. Do not send original art work, x-ray films, or ECG tracings. Glossy print photographs are preferred, for good black and white contrast is essential.

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A brief case presentation of special interest in the form of a Letter to the Editors will be considered for publication. All such letters should be sent to Dr. Frederick P. Zuspan.

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Nearly everybody believed that a very low dose oral contraceptive automatically meant a high incidence of breakthrough bleeding.

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averaged only 3.9% breakthrough bleeding in total cycles.

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Minor side effects—even breakthrough bleeding—have been remarkably infrequent

In clinical trials the incidence of breakthrough bleeding in the initial cycle was only nine percent. And even that low incidence decreased to an average of only 3.9 percent as cycles progressed.

Incidence of Breakthrough Bleeding in Premarketing Clinical Trials

Cycle	1	3	6	12	Total Cycles
					11,085
Breakthrough Bleeding	9.0%	3.5%	2.7%	2.6%	3.9%

Acceptance has been excellent

The low incidence of minor side effects has resulted in ready acceptance of LO/OVRAL by the great majority of patients. Patient complaints have been infrequent. Only eight percent of women participating in clinical trials discontinued LO/OVRAL because of common side effects.

Steroidal balance and norgestrel make the difference

Although total steroids in LO/OVRAL are reduced by 40 percent, the time-proved progestogen-estrogen ratio of OVRAL** is retained. This, together with norgestrel's distinctive qualities, is largely responsible for the patient comfort and acceptance experienced to date. Unlike other progestogens in current use, norgestrel is non-estrogenic, is apparently not converted to estrogen *in vivo* and closely resembles natural progesterone in its progestogenic activity.

Simple to take

The dosage regimen with LO/OVRAL is convenient and easy to follow: 3 weeks on, 1 week off. The attractive, uncomplicated Pilpak® dispenser helps patients follow the right dosage schedule.

*Serious as well as minor adverse reactions have been reported following the use of all oral contraceptives. The physician should remain alert to their earliest manifestations and in the event of serious adverse reactions should avoid any oral contraceptives. *Special note, contraindications, warnings, precautions and adverse reactions* in the accompanying IN BRIEF should be carefully considered.

**OVRAL® each tablet contains 0.5 mg. norgestrel with 0.05 mg. ethinyl estradiol, Wyeth.

Important information on next page.

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Low dose oral contraceptive.

IN BRIEF:

SPECIAL NOTE: Oral contraceptives have been marketed in the United States since 1960. Reported pregnancy rates vary from product to product. The effectiveness of the sequential products appears to be somewhat lower than that of the combination products. Both types provide almost completely effective contraception.

An increased risk of thrombotic, thromboembolic and cerebral-vascular disease associated with the use of hormonal contraceptives has been shown in studies conducted in both Great Britain and the United States. Other risks, such as those of elevated blood pressure and reduced tolerance to carbohydrates, have been reported, and appropriate tests should be conducted to monitor these during oral-contraceptive therapy. Liver disease has also been reported, and the physician should be alert to its earliest manifestations.

Long-term administration of both natural and synthetic estrogens in subprimate animal species in multiples of the human dose increases the frequency for some animal carcinomas. These data cannot be transposed directly to man. The possible carcinogenicity due to the estrogens can neither be affirmed nor refuted at this time. Close clinical surveillance of all women taking oral contraceptives must be continued.

INDICATIONS: For oral contraception.

CONTRAINDICATIONS: 1. Thrombophlebitis, thromboembolic disorders, cerebral-vascular disease, or a past history of these conditions; 2. Markedly impaired liver function; 3. Known or suspected carcinoma of the breast; 4. Known or suspected estrogen-dependent neoplasia; 5. Undiagnosed abnormal genital bleeding; 6. Known or suspected pregnancy.

WARNINGS: 1. The physician should be alert to the earliest manifestations of thrombotic and thromboembolic disorders, thrombophlebitis, cerebrovascular disorders including hemorrhage, pulmonary embolism and retinal thrombosis. Should any of these occur or be suspected, the drug should be discontinued immediately.

Retrospective studies of morbidity and mortality in Great Britain and studies of morbidity in the United States have shown a statistically significant association between thrombophlebitis, pulmonary embolism and cerebral-vascular disease, occlusive or hemorrhagic, and the use of oral contraceptives. There have been three principal studies in Great Britain[1-3] leading to these conclusions and three[4-7] in this country. The estimate of the relative risk of thromboembolism in the study by Vessey and Doll[3] was about sevenfold, while the United States studies found relative risks of 4.4 to 11, meaning that the users are several times as likely to undergo thromboembolic disease without evident cause as non-users.

In May, 1974, the Royal College of General Practitioners in England[8] issued an interim report of its continuing large-scale prospective study comparing a user group to a non-user group. This study in its interim analysis states: "A statistically significant higher rate of reporting of cerebrovascular accidents in Takers is evident, but the numbers are too small to justify an estimation of the degree of risk." The study also reported a higher incidence of superficial and deep vein thrombosis in users as compared to non-users.

The Sartwell study[4] indicated that the risk did not persist after discontinuation of administration. Both the Sartwell and the Royal College study indicated that the degree of risk was not associated with duration of treatment.

In a collaborative American study[5,6] of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the relative risk of thrombotic stroke was 4.1 to 9.5 times greater in users than in non-users. A comparable estimate for hemorrhagic stroke was 2.0.

None of the American studies was designed to evaluate a difference between products. However, the Sartwell study[4] suggested that there might be an increased risk of thromboembolic disease in users of sequential products.

Other retrospective studies[9,10] have reported an increased risk of post-surgery thromboembolic complications in oral-contraceptive users. It has been recommended that therapy be discontinued at least one month prior to elective surgery.

2. Discontinue oral-contraceptive medication if there is: gradual or sudden, partial or complete loss of vision; proptosis or diplopia; onset or aggravation of migraine or development of headache of a new pattern which is recurrent, persistent or severe; papilledema; or any evidence of retinal-vascular lesions. 3. Fetal abnormalities have been reported to occur in the offspring of women who have taken progestogens and/or estrogens during pregnancy.[11,12] The safety of LO/OVRAL® in pregnancy has not been demonstrated. Pregnancy should be ruled out before initiating or continuing the contraceptive regimen. Pregnancy should always be considered if withdrawal bleeding does not occur. 4. A small fraction of the hormonal agent in oral contraceptives has been identified in the milk of mothers receiving these drugs. The long-range effect to the nursing infant cannot be determined at this time. 5. Hepatic lesions (adenomas, hepatomas, hamartomas, regenerating nodules, etc.), occasionally fatal, have been reported in women on oral contraceptives. Such lesions may present as an abdominal mass or with the signs and symptoms of an acute abdomen. These lesions should be considered if the patient has abdominal pain or evidence of intra-abdominal bleeding. Such lesions have been reported in short-term as well as long-term users of oral contraceptives.

PRECAUTIONS: 1. A thorough history and physical examination should be performed before prescribing oral contraceptives and periodically during their administration and should include special reference to breasts and pelvic organs including Papanicolaou smears, since estrogens have been known to produce tumors, some of them malignant, in five species of subprimate animals. 2. Endocrine and possibly liver-function tests may be affected by treatment with LO/OVRAL® Therefore, if such tests are abnormal in a patient taking LO/OVRAL® it is recommended that they be repeated after the drug has been withdrawn for two months. 3. Under the influence of estrogen-progestogen preparations, pre-existing uterine fibromyomata may increase in size. 4. Because these agents may cause some degree of fluid retention, conditions which might be influenced by this factor, such as epilepsy, migraine, asthma, cardiac or renal dysfunction, require careful observation. 5. In breakthrough bleeding, and in all cases of irregular bleeding per vaginam, nonfunctional causes should be borne in mind. In undiagnosed bleeding per vaginam, adequate diagnostic measures are indicated. 6. Patients with a history of psychic depression should be carefully observed and the drug discontinued if the depression recurs to a serious degree. 7. Any possible influence of prolonged LO/OVRAL® therapy on pituitary, ovarian, adrenal, hepatic or uterine function awaits further study. 8. A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. The mechanism of this decrease is obscure. For this reason, diabetic patients should be carefully observed while receiving LO/OVRAL® therapy. 9. The age of the patient constitutes no absolute limiting factor, although treatment with LO/OVRAL® may mask the onset of the climacteric. 10. The pathologist should be advised of LO/OVRAL® therapy when relevant specimens are submitted. 11. Susceptible women may experience an increase in blood pressure following administration of contraceptive steroids. 12. Cholestatic jaundice has been reported in users of oral contraceptives. If this occurs, LO/OVRAL® should be discontinued. This condition is more likely to occur in patients who have experienced cholestatic jaundice of pregnancy. Patients with a history of cholestatic jaundice of pregnancy should be carefully observed during LO/OVRAL® therapy.

ADVERSE REACTIONS OBSERVED IN PATIENTS RECEIVING ORAL CONTRACEPTIVES: A statistically significant association has been demonstrated between use of oral contraceptives and the following serious adverse reactions: thrombophlebitis, pulmonary embolism, cerebral thrombosis and hemorrhage, gall bladder disease.

Although available evidence is suggestive of an association, such a relationship has been neither confirmed nor refuted for the following serious adverse reactions: neuro-ocular lesions, e.g., retinal thrombosis, optic neuritis and hepatic lesions with or without intra-abdominal bleeding.

The following adverse reactions are known to occur in patients receiving oral contraceptives: nausea, vomiting, gastrointestinal symptoms (such as abdominal cramps and bloating), breakthrough bleeding, spotting, change in menstrual flow, amenorrhea during and after treatment, edema, chloasma or melasma, which may persist, breast changes: tenderness, enlargement and secretion, change in weight (increase or decrease), changes in cervical erosion and cervical secretions, suppression of lactation when given immediately post-partum, cholestatic jaundice, migraine, rash (allergic), rise in blood pressure in susceptible individuals, mental depression.

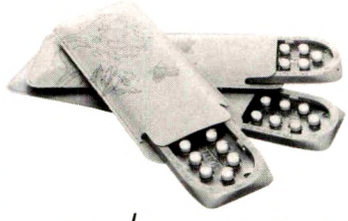
Although the following adverse reactions have been reported in users of oral contraceptives, an association has been neither confirmed nor refuted: anovulation post treatment, which tends to occur more frequently in women with a history of menstrual disorders, premenstrual-like syndrome, changes in libido, changes in appetite, cystitis-like syndrome, headache, intolerance to contact lenses, nervousness, dizziness, fatigue, backache, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, itching and vaginitis.

The following laboratory results may be altered by the use of oral contraceptives: hepatic function; increased sulfobromophthalein retention and other tests; coagulation tests; increase in prothrombin, Factors VII, VIII, IX and X; decrease in antithrombin III; increase in platelet aggregability; thyroid function: increase in PBI, and butanol extractable protein-bound iodine and decrease in T^3 uptake values; metyrapone test; and pregnanediol determination.

NOTE: For more specific details on LO/OVRAL, refer to package insert.

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OBSTETRICS

Termination of pregnancy with double prostaglandin impact

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With the "double PG impact" (DPGI) technique in 100 sedated first- and second-trimester volunteers, pregnancy was terminated successfully in 99 with a total extraovular $PGF_{2\alpha}$ dose of 19.0 ± 1.5 mg. in 16.9 ± 0.9 hours. Two thirds of the patients received only the initial dose of 11.6 ± 0.5 mg. of PG and aborted in 12.9 ± 0.7 hours without requiring supplemental treatment. Only mild and transient side effects and 14 indicated curettages were recorded. Excepting one gravida (who failed to abort), plasma progesterone in 99 patients decreased at 3 hours after DPGI from 32.5 ± 1.7 to 22.7 ± 1.2 ng. per milliliter ($P < 0.001$). The rate of progesterone withdrawal and IAT showed a close relationship and the first-trimester patients aborted at a lower progesterone level than the second-trimester patients ($P < 0.001$).

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AS LONG AS pregnancy termination is a legal procedure, leading medical centers are medically obliged to develop advanced methods of management in the best interest of the patients. During several decades the accepted procedures were (1) dilatation and sharp curettage (D & C) for interrupting first-trimester and

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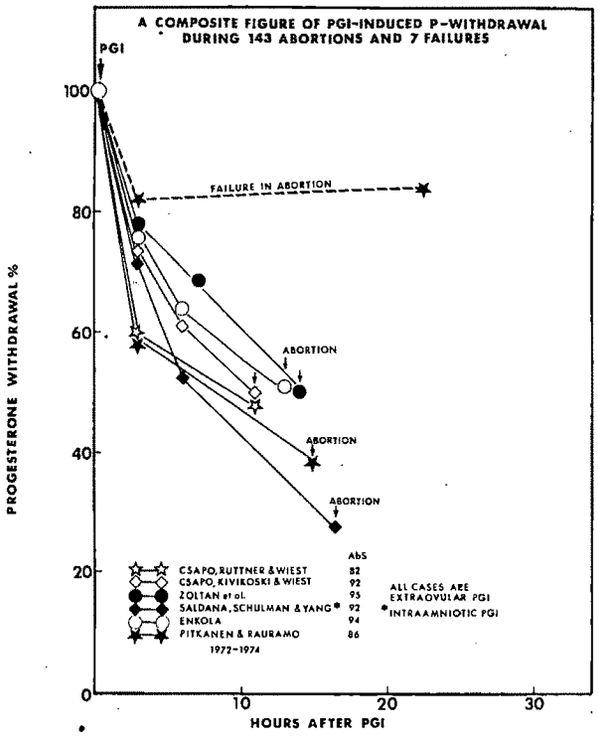


Fig. 1. A summary of the effect of PGI on plasma P levels. For description of this and subsequent figures, see Text.

(2) hysterotomy for terminating second-trimester pregnancies. In most institutions sharp curettage has currently been replaced by suction and hysterotomy by the hypertonic saline method. In the hands of trained specialists, these procedures improved postconceptional therapy.

Statistical evidence showed, however, that in contrast to spontaneous abortions, rapid surgical dilatation affects the structural integrity of the cervix and that "incompetent os" predisposes to premature labor.[1] It also revealed that occasional technical failures in the intra-amniotic administration of hypertonic saline increase the risks of the "salting-out" technique.[2] Therefore, the early reports that prostaglandins (PGs) terminate pregnancy, without harmful consequences, generated considerable interest.[3]

Subsequent trials, however, in which PGs were administered by intravenous or extraovular infusions or by repeated injections, with the intent of stimulating the normal pregnant uterus, have failed to yield clinically acceptable results. The *refractory* uterus did not promptly respond to PGs with cyclic intrauterine pressure (IUP) of that magnitude which was measured several hours after effective PG treatment. The initial response was an unphysiologic and sustained contracture, which changed to cyclic IUP only gradually with the progress of *time*. To increase efficacy of prolonged administration the dose of PGs had to be increased, which resulted in unacceptable side effects. Thus the therapeutic principle "PG-impact" (PGI) had been considered as an alternative approach,[1] with the intent of provoking a *regulatory imbalance,* before attempting myometrial stimulation for the promotion of clinical progress.

The PGI was effective in terminating pregnancy[4] regardless of whether the administration of the drug was intra-amniotic or extraovular. The intra-amniotic method, however, had the disadvantage of transabdominal puncture (in local anesthesia), occasional bloody taps, prolonged instillation-abortion time (IAT, resulting from the initial dilution of the drug by the amniotic fluid), and repeated side effects due to the protracted adsorption of PG. Extraovular PGI had the advantages of transcervical administration, reduced dosage, and short-term side effects (due to rapid elimination of the drug) which could be reduced by sedation. Occasional failures in the effective delivery of PGI limited the broad therapeutic application of this method, however.

To improve the efficacy of PGI, the clinical benefits of the modified technique "double prostaglandin impact" (DPGI) had been considered. The rationale of this modification was based on earlier results (for references and summary, see Fig. 1 and Southern, Csapo, Saldana and their co-workers[4–9], which showed that the single PGI is effective if it provokes a regulatory imbalance, resulting from progesterone withdrawal (Pw), which *converts* the refractory uterus to a reactive organ.[5] Once this conversion had been provoked by Pw, threshold decreased and the uterus readily responded to both endogenous and exogenous stimulation, promoting clinical progress in abortion.[1, 4–9] Therefore, the possibility has been considered that by amplifying the initial effect of PGI, through direct and rapid contact between the drug and the uterus at two points (rather than only one), DPGI (at the same dose level) might increase the efficacy of PGI. In the present study the validity of this premise has been examined.

Study patients and methods

Selection of study patients. After thorough physical and laboratory workup, staff physicians presented their cases to the committee as part of the clinical routine of the department. If the committee approved legal abortion, the alternative techniques were explained to the patients. The obstetrically normal gravidas who formed the study group were those who consented (in writing) both to the use of the "prostaglandin-impact" (PGI) method and to a su-

perimposed academic study of the clinical course of the abortion. The research staff was then requested to recommend a delivery system and a dose which promised the most favorable clinical outcome and to conduct observations of academic interest which promised future therapeutic benefits.

Design of the study. The following considerations were made. While PGE2 was found to be a more effective abortifacient than PGF2α on a per-weight basis, this higher efficacy was not reflected in a reduction of side effects. Synthetic PG analogues were claimed to be more efficacious and acceptable than the natural compounds, but this claim had not been verified.[6] It seemed preferable, therefore, to use the marketed compound PGF2α* and focus interest on improving its delivery to the target organs.

Earlier examinations[1, 4–9] of the efficacy of PGI revealed (Fig. 1) that this technique is effective in terminating pregnancy, regardless of the gestational age of the patients (up to the legal cutoff point of 20 weeks' gestation), provided it provoked rapid and continued Pw. Thus the technique of "double PGI" (DPGI) was conceived and adopted (Protocol No. 5026) with the *intent of provoking effective Pw* more predictably. This method involves the placement, into the left and right sides of the extraovular space, two PG doses which together do not exceed the single dose, previously delivered at one side only. By an extended action with the uterus, at two direct contact points (rather than one), DPGI was expected to provoke Pw more predictably than did PGI.

Procedure. After thorough vaginal preparation and sedation (50 mg. of pethidine, 10 mg. of diazepam, and 0.4 mg. of atropine), a semi-stiff catheter with a flexible tip was introduced transcervically into the left side of the lower pole of the extraovular space under sterile precautions. When in position, the tip of the catheter was moved upward to at least 5 cm. or higher from the internal os. Through this catheter first-trimester patients received an initial dose of 5 mg. of PGF2α and through a second catheter (inserted into the right side of the extraovular space) another 5 mg. After the instillation of each 5 mg. of PGF2α the catheter was withdrawn. Through a similar procedure, second-trimester patients received double doses, 2 × 10 = 20 mg. of PGF2α. Treatment through each catheter lasted for about 5 minutes.

Before, during, and after DPGI, the vital signs of the patients and side effects were recorded. If during PG administration the side effects exceeded the acceptable range, treatment was discontinued.

Patients who aborted, or had advanced cervical dilatation in 8 hours, received no further treatment. If, however, clinical progress was slow, a second dose of PGF2α was administered extraovularly at 8 hours after DPGI. First-trimester pregnant patients received 5 mg. and second-trimester patients received 10 mg. of PGF2α. Occasionally, when this supplemental treatment did not promote distinct clinical progress, an additional dose was given extraovularly or 50 m.U. of oxytocin per minute were administered intravenously. If the fetal membranes (of second-trimester patients) were ruptured accidentally during catheter placement, the initial dose of 20 mg. of PGF2α was delivered nevertheless. The intra-amniotic (rather than extraovular) delivery of the compound was noted, however, to allow retrospective comparison between the efficacy of extraovular and intra-amniotic (transcervical) administration.

Since, in all earlier trials, the effect of PGI on the resting and cyclic intrauterine pressure (RP and IUP) had been monitored throughout the instillation-abortion time (IAT) and the relationship between PGI, RP, IUP, and clinical progress had been established,[1, 4–9] in this study IUP was not recorded. However, blood samples were collected before, at 3 and 10 hours after DPGI, at the time when the fetus was aborted, and 12 hours after the completion of abortion. While it had been demonstrated[4–9] that the therapeutic success of PGI depends on the rate and degree of Pw it provokes (Fig. 1), nevertheless the present quantitation of Pw seemed desirable, since the efficacy and mode of action of a modified procedure (DPGI) had been the subject of study.

After the abortion of the fetus, up to 10 hours was allowed for the spontaneous delivery of the placenta, unless strong bleeding demanded instant evacuation of the uterus by curettage. If the placenta was detached from the uterine wall, it was removed from the cervix or vagina by abortion forceps. Since, in first-trimester gravidas, it is difficult to determine the completeness of abortion, and since the postabortion course was of interest, the patients were divided (post partum) into two groups. One group was subjected to diagnostic curettage (to determine whether or not placental residue was present) and the other group was not. Careful notes were made throughout the study about the patients' temperature, WBC, cervical effacement and dilatation, and the onset and character of the subsequent spontaneous menstrual period.

To assess clinical success, the degree of abortion of each patient was graded by an abortion score (AbS):

*The authors are grateful to Drs. E. M. Southern and G. D. Gutknecht of the Upjohn Company for the generous supply of PGF2α.

Table I. The effect of DPGI in 99 patients*

No. of cases	Age (yr.)	Para No.	Gest. age (wk.)	PGF2α (mg.) Initial	PGF2α (mg.) Total	IAT (hr.)	Abortion score
First-trimester pregnant:							
67	25.2 ±0.8	1.0 ±0.1	9.4 ±0.2	10.0 ±0.0	12.7 ±0.5	17.0 ±1.2	98.2 ±1.0
Second-trimester pregnant:							
32	21.0 ±1.1	0.6 ±0.2	16.3 ±0.4	18.7 ±0.6	33.5 ±3.5	16.7 ±1.3	90.3 ±3.0
Total:							
99	24.4 ±0.7	0.9 ±1.1	11.6 ±0.4	12.7 ±0.5	19.0 ±1.5	16.9 ±0.9	95.6 ±1.3
Extraovular DPGI only†:							
91	24.7 ±0.7	1.0 ±0.1	11.0 ±0.4	12.2 ±0.4	16.4 ±1.1	16.1 ±1.0	96.5 ±1.2

*All values are means ± S.E. in this and all subsequent tables.

†The eight cases of intra-amniotic PGI (due to accidental rupture of fetal membranes) are excluded.

Table II. The effect of DPGI in 67 first trimester pregnant patients

No. of cases	Age (yr.)	Para No.	Gest. age (wk.)	PGF2α (mg.) Initial	PGF2α (mg.) Total	IAT (hr.)	Abortion score
Initial DPGI only:							
44	26.9 ±1.0	1.3 ±0.2	9.0 ±0.2	9.9 ±0.1	9.9 ±0.1	12.8 ±0.7	100.0 ±0.0
Initial and supplementary DPGI:							
23	23.2 ±1.0	0.5 ±0.2	10.0 ±0.3	10.0 ±0.0	18.0 ±0.8	25.1 ±2.6	94.8 ±2.9

100 = complete abortion, 80 = retained placental residue, 60 = complete placental retention, 40 = 2 cm. cervical dilatation. Complications during and after DPGI were recorded and treated. Specific attention was directed toward the pain response of the sedated patients to DPGI and to symptoms of infection before, during, and after abortion, of course, patients with cervicitis had first been treated, or excluded from the study.

In the sequentially collected plasma samples, progesterone (P) and estradiol-17β (E_2) had been determined by radioimmunoassays.[8] All information was placed on computer sheets and subjected to statistical analysis. In examining and tabulating the results, interest was directed specifically toward those parameters which affected the instillation-abortion time (IAT) since, in comparison with the surgical procedures, the major drawback of the pharmacologic methods seemed to be the prolongation of the IAT, demanding lengthy medical supervision and hospitalization.

Results

Table I describes the clinical outcome of 99 pregnancy terminations in patients 6 to 20 weeks pregnant, with the extraovular "double prostaglandin impact" (DPGI) technique. The single case of treatment failure will be described separately. All 67 first-trimester gravidas, who (at the mean ± S.E. gestational age of 9.4 ± 0.2 weeks) received an initial dose of 10.0 ± 0.0 (and a total dose of 12.7 ± 0.5) mg. of PGF2α, aborted in 17.0 ± 1.2 hours with an exceptionally high abortion score (AbS) of 98.2 ± 1.0. All 32 second-trimester gravidas, who (at 16.3 ± 0.4 weeks of gestation) received an initial dose of 18.7 ± 0.6 (and a total dose of 33.5 ± 3.5) mg. of PGF2α, aborted in 16.7 ± 1.3 hours with a high AbS of 90.3 ± 3.0. Thus the 99 study patients, who (at 11.6 ± 0.4 weeks of gestation) received an initial dose of 12.7 ± 0.5 (and a total dose of 19.0 ± 1.5) mg. of PGF2α, aborted in 16.9 ± 0.9 hours with an exceptionally high AbS of 95.6 ± 1.3. Out of 100 gravidas, only one (15 weeks pregnant) multipara failed to abort (in 48 hours), but even this patient progressed to a 2 cm. dilatation after having received a total dose of 50 mg. of PGF2α.

Apparently,[4–9] DPGI yielded better clinical results than PGI (Fig. 1). During catheter placement, the fetal membranes of eight (out of the 32 second-trimester) patients were ruptured accidentally. These accidents represent technical (rather than treatment) failures since, after the membranes were ruptured, PGI was delivered intra-amniotically (rather than extraovularly). In these eight patients abortion was induced without failure. Since the total PG requirement and IAT of these eight gravidas were excessive, however, their removal from the study group improved the outcome of the 91 extraovular cases regarding the (1) total PGF2α dose (16.4 ± 1.1 mg.), (2) IAT (16.1 ± 1.0 hours), and (3) AbS (96.5 ± 1.2), as illustrated by Table I.

Even if the statistical evidence is considered,[1] however suggesting that repeated D & C predisposes to prematurity, surgical rather than pharmacologic pregnancy termination might still be preferred, if the IAT after DPGI is as long as 17.0 ± 1.2 hours (as was the case in the 67 first-trimester pregnant patients listed in Table I). Thus the reasons behind this lengthy IAT were scrutinized, as illustrated by Table II. The results showed that those 44 (out of 67) gravidas who (at 9.0 ± 0.2 weeks of gestation) required only the initial dose of 9.9 ± 0.1 mg. of PGF2α, without supplemental treatment, aborted in 12.8 ± 0.7 hours with a maximal AbS of 100.0 ± 0.0. In contrast, those 23 (out of 67) gravidas who (at 10.0 ± 0.3 weeks of gestation) re-

Table III. Changes in circulating plasma progesterone levels induced by the double prostaglandin impact

No. of cases	Gest. age (wk.)	Total PGF2α (mg.)	IAT (hr.)	Progesterone (ng./ml.) after DPGI (hr.)			
				0	3	10	Ab
Successful extraovular DPGI:							
91	11.0 ±0.4	16.4 ±1.1	16.1 ±1.0	30.7 ±1.7	20.8 ±1.1	16.1 ±0.9	13.4 ±0.8
Successful intra-amniotic PGI (accidental membrane rupture):*							
8	17.3 ±0.4	59.4 ±6.8	26.3 ±1.1	54.0 ±5.8	44.0 ±4.5	38.3 ±4.9	28.1 ±6.8
Unsuccessful extraovular DPGI†							
1	15	50	48	51	41	39	39
Total:							
100	11.6 ±0.4	19.0 ±1.5	16.9 ±0.9	32.5 ±1.7	22.7 ±1.2	17.8 ±1.1	14.6 ±1.0

*PGI delivered intra-amniotically because the membranes ruptured accidentally.

†One case of failure; pregnancy terminated by hypertonic saline.

Table IV. The relationship between gestational age, prostaglandin dose, progesterone levels, and instillation-abortion time

No. of cases	Gest. age (wk.)	Total PGF2α (mg.)	IAT (hr.)	Progesterone (ng./ml.) after DPGI (hr.)			
				0	3	10	Ab
First trimester:							
67	9.4 ±0.2	12.7 ±0.5	17.0 ±1.2	24.2 ±0.9	17.7 ±0.8	13.0 ±0.8	10.7 ±0.7
Second trimester:							
24	16.0 ±0.5	25.2 ±2.3	13.5 ±1.1	45.6 ±2.9	30.9 ±2.8	23.5 ±1.9	20.3 ±1.5
Initial DPGI only, first and second trimesters:							
58	10.5 ±0.4	11.6 ±0.5	12.9 ±0.7	28.6 ±1.6	19.0 ±1.1	14.1 ±0.1	12.3 ±1.0
Initial + supplemental DPGI, first and second trimesters:							
33	12.1 ±0.7	22.8 ±1.6	21.9 ±2.1	32.1 ±2.8	23.5 ±2.2	18.8 ±1.6	14.9 ±1.4

quired a total dose of 18.0 ± 0.8 mg. of PGF2α, had 25.1 ± 2.6 hours long IAT (with a high AbS of 94.8 ± 2.9, however).

This paradox, that gravidas (34 per cent) who received an average second dose of 8 mg. of PGF2α (supplementing the initial 10 mg.) had an IAT twice as long as that of those patients (66 per cent) who required only the initial (10 mg.) dose, had to be explained. Further scrutiny of the results revealed that while the "fast" aborters were para 1.3 ± 0.2 and the "slow" aborters para 0.5 ± 0.2, parity per se did not explain the difference in the IAT of these two groups of patients. The various retabulations of results indicated that nulliparity only prolongs the IAT in excess of 30 per cent beyond that of the multiparous, because the nulliparous cervix presents an obstacle against the effective transcervical and extraovular delivery of PGI. Proper consideration of this clinical reality is mandatory, since the parity of the patients is outside the control of the attending physician, while the delivery system used by him could be further improved.

If the prolongation of the IAT is only partly due to nulliparity (in both first- and second-trimester patients) and partly to failures in the effective delivery of DPGI, then the decrease in plasma progesterone (P) levels can be expected to be slower in the "slow" than in the "fast" aborters. This premise is based on earlier studies,[4-9] which showed (Fig. 1) a correlation between the rate of P withdrawal (Pw), the length of the IAT, and the therapeutic success in PG-induced abortions.

The changes in P levels induced by extraovular DPGI (or intra-amniotic PGI) are shown in Table III, providing useful information about the control of the IAT. In 91 cases, extraovular DPGI provoked a 32 per cent Pw as early as 3 hours after treatment ($P < 0.001$) and 56 per cent Pw at 16.1 ± 1.0 hours ($P < 0.001$), when patients aborted. In contrast, intra-amniotic PGI in eight gravidas (correcting technical failure with attempted DPGI) provoked a 19 per cent Pw at 3 hours after treatment ($P > 0.05$) and a 48 per cent Pw at 26.3 ± 1.1 hours ($P < 0.05$), when these eight patients aborted. Thus, in spite of the considerably greater total PGF2α dose, in cases of intra-amniotic (59.4 ± 6.8 mg.) rather than extraovular (16.4 ± 1.1 mg.) treatments, the rate of Pw was faster and the IAT shorter in the extraovular group. Furthermore, one patient, whose Pw did not continue after the initial 20 per cent decrease, in spite of her exposure to a total dose of 50 mg. of PGF2α, did not abort in 48 hours. Of additional interest is the finding (Table III), already recognized in earlier studies, that the P value at which the patients aborted is dependent on gestational age.

Fig. 2 describes the relationship between the rate of Pw and the IAT, as well as between the degree of Pw and the success of PG treatment. It illustrates that ~50 per cent Pw precedes successful pregnancy termination with the DPGI technique. Since this relationship between Pw and IAT appears to be the controlling mechanism of the success in this form of pharmacologic pregnancy termination, the results will be further examined.

Table IV illustrates that DPGI provoked in both

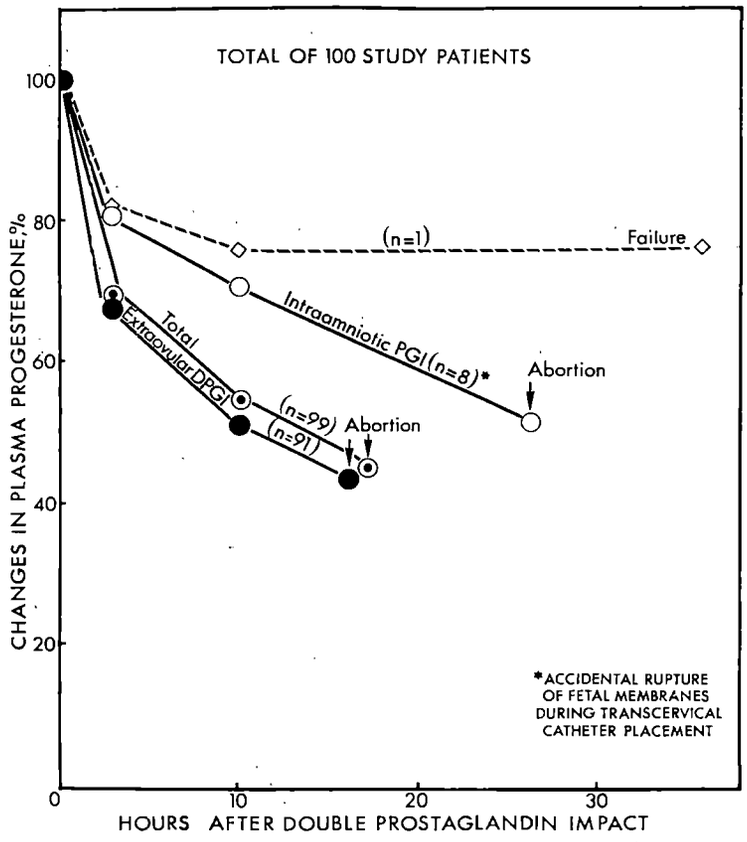


Fig. 2. The rate and degree of Pw provoked by DPGI in 100 patients.

first- and second-trimester pregnant patients significant ($P < 0.001$) Pw as early as 3 hours after treatment (27 and 32 per cent respectively). The degree of Pw at the time of abortion was similar in both groups (56 and 55 per cent respectively). Critical abortion values were reached in 13.5 ± 1.1 hours in the second- and 17.0 ± 1.2 hours in the first-trimester pregnant patients. It is of further interest that while the degree of Pw was similar in the first- and second-trimester patients, the 9.4 ± 0.2 weeks pregnant women aborted at a P level of 10.7 ± 0.7 ng. per milliliter and the 16.0 ± 0.5 weeks pregnant patients at 20.3 ± 1.5 ng. per milliliter ($P < 0.001$). This finding illustrates the critical dependence of the P value at which pregnancy is terminated on gestational age.

Of considerable interest is the additional finding (Table IV) that the group of 58 (first- and second-trimester) gravidas who required only the initial 11.6 ± 0.5 mg. of DPGI for a 58 per cent Pw in 12.9 ± 0.7 hours aborted at this time, whereas those 33 (first- and second-trimester) patients who required supplemental treatment and a total dose of 22.8 ± 1.6 mg. of PGF2α for a 51 per cent Pw in 21.9 ± 2.1 hours aborted at that time. Evidently, the rate of Pw, not the total PGF2α dose, controlled the IAT. This finding is of considerable interest, since it suggests that the clinical success of provoking abortion with a short IAT is determined by the effect of the *initial* PG impact on the P levels.

This conclusion—that fast abortions demand a rapid removal of the P excess (which protects pregnancy)—exposed a new and *quantitative therapeutic endpoint* in the management of pregnancy termination. Detailed examination of the changes in the P levels of 67 first-trimester pregnant patients verified this conclusion. Fig. 3 illustrates that, regardless of how the 67 first-trimester gravidas were grouped (e.g., on the basis of parity or total PGF2α dose), they aborted when 45 to 60 per cent of their P excess had been removed by the effect of DPGI. The absolute P values at which the different groups of patients aborted ranged from 9.5 ± 1.0 to 13.4 ± 1.7 ng. per milliliter. This already narrow range of P was actually narrower, since the patients who aborted at the P level of 9.5 ± 1.0 ng. per milliliter were only 8.7 ± 0.3 weeks pregnant, whereas those who did at 13.4 ± 1.7 ng. per milliliter were 9.5 ± 0.3 weeks pregnant. The effect of gestational age on the P value at which the patients abort having

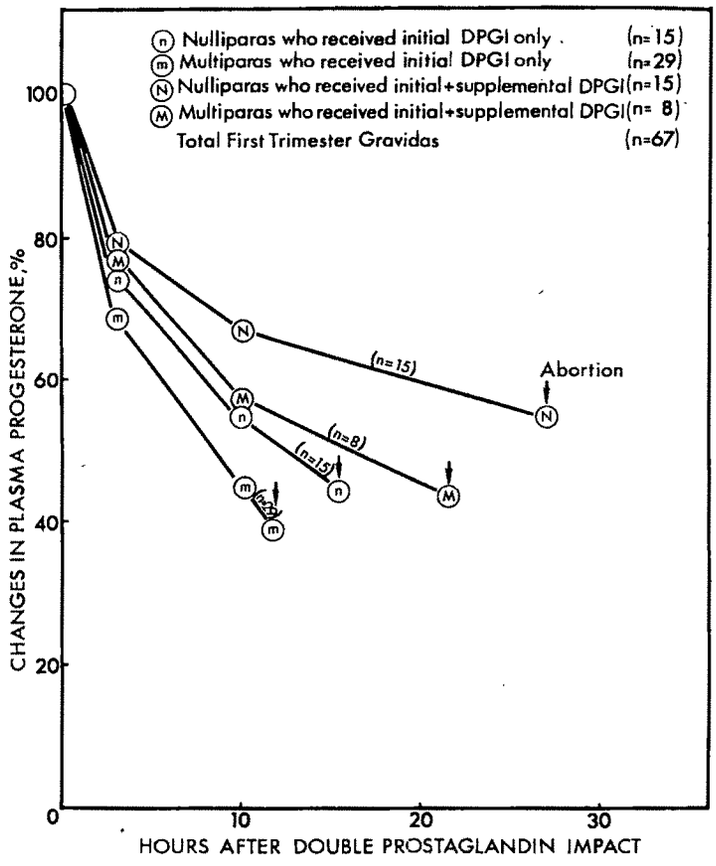


Fig. 3. The rate and degree of Pw in 67 first-trimester patients.

already been demonstrated in Table IV, this finding shows the reality of the "abortion P value" as a quantitative endpoint in PG-induced abortions.

Fig. 3 also illustrates that, while multiparas aborted earlier than nulliparas, and gravidas who required only the initial DPGI aborted earlier than those who required supplemental DPGI, nevertheless the IAT was controlled by the rate of Pw. This important point was re-emphasized by the finding that nulliparas, who required only the initial DPGI for rapid Pw (Group: N) aborted several hours before those multiparas did who required supplemental DPGI to reach abortion P value (Group: M). Apparently, regardless of how the groups were formed (whether on the basis of gestational age, parity, or total PGF2α received), the IAT continued to be controlled by the rate of Pw. Thus future clinical trials with PGs should be so designed that the delivery system of the drug guarantees rapid Pw to the critical abortion value.

The results presented so far demonstrate that (1) Pw to a critical value precedes DPGI-induced abortion, (2) the absolute value to which P has to decrease depends on gestational age, and (3) the rate of Pw controls the IAT.

Fig. 4 describes the clinical characteristics and steroid profiles of the "fast" and "slow" aborters. Disregarding parity (which has been discussed already), the 63 fast aborters were different from the 36 slow aborters in that they (1) received less PGF2α (15.3 ± 1.2 rather than 26.1 ± 3.4 mg.; $P < 0.01$); (2) had a shorter IAT (11.5 ± 0.4 rather than 26.5 ± 1.5 hours; $P < 0.001$); (3) reached their critical P value earlier (in 11.5 ± 0.4 rather than in 26.5 ± 1.5 hours; $P < 0.001$).

These and the additional findings that the fast aborters (1) outnumbered by far the slow aborters (64 per cent), (2) required only the initial DPGI in most instances (71 per cent), and (3) reached critical P value and aborted in 11.5 ± 0.4 hours without supplemental PG stimulation provided evidence that the delivery system DPGI is an effective clinical procedure for the termination of both first- and second-trimester pregnancies.

The additional considerations that: (1) the fast aborters were para 1.2 ± 0.1, whereas the slow aborters were para 0.4 ± 0.1, and (2) 64 per cent of the slow aborters required supplemental PG treatment and, in spite of this supportive therapy, only reached critical P value and aborted in 26.5 ± 1.5 hours, suggest that the

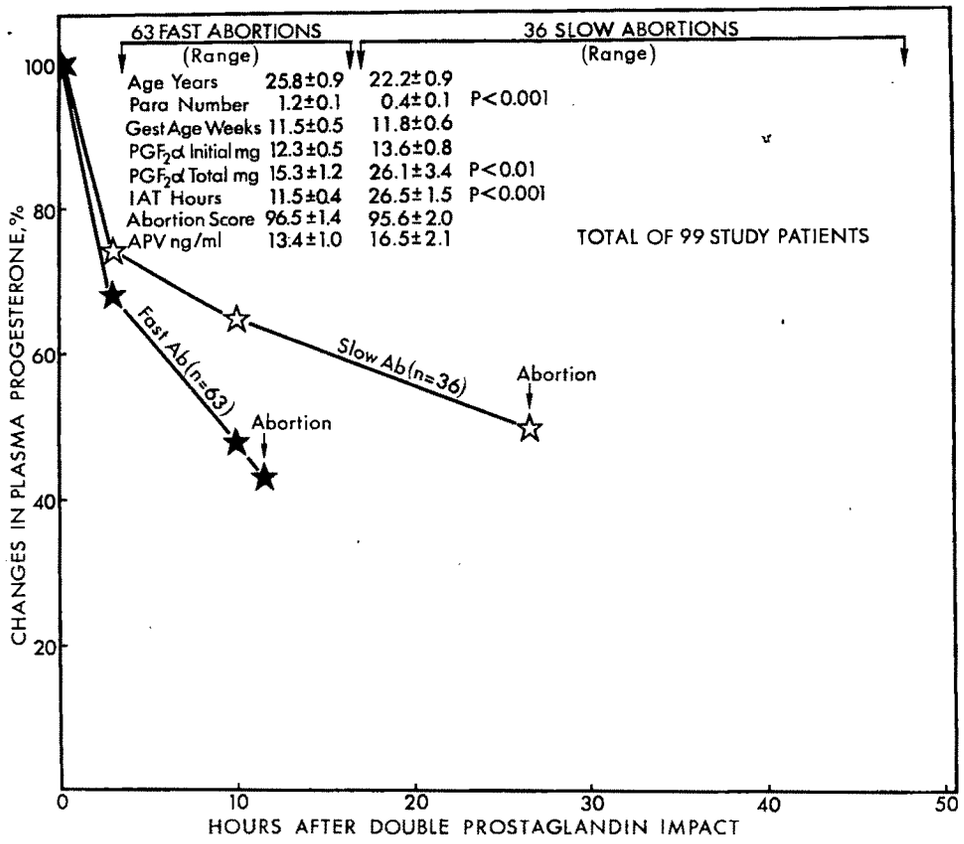


Fig. 4. The rate and degree of Pw in 63 "fast" and 36 "slow" aborters.

effective transcervical delivery of DPGI is occasionally hindered by the nulliparous (or otherwise unfavorable) *cervix.* Since this technical obstacle (being usually recognized after the initial failure in clinical progress) can be corrected only by repeated supplemental treatment, the IAT becomes prolonged.

The elimination of this group (the slow aborters) should be given high priority in future research. The results also showed (Fig. 4) that the slow aborters (who received in 64 per cent of the cases supplemental PG treatment) had slightly less Pw and aborted at a slightly higher P value than the fast aborters. While these differences are not significant statistically, they suggest that (within limits) a lesser degree of Pw is demanded for abortion if the uterus is stimulated continuously by repeated PG treatment or oxytocin infusion.

The results obtained in the 24 second-trimester pregnant patients (who received extraovular DPGI) substantiated the conclusions based on the study of the 67 first-trimester gravidas. Even those eight second-trimester patients who (after accidental rupture of the fetal membranes) received intra-amniotic PGI showed the relationship between Pw, IAT, and abortion. The small total number of second-trimester gravidas did not permit statistical analysis in subgroups.

The rates of cervical effacement and dilatation are informative clinical parameters. Since the transcervical passage of the small uterine contents of first-trimester pregnant patients requires only moderate cervical dilatation (which is difficult to estimate), only the cervical dilatation of the second-trimester gravidas could be correlated with Pw.

Fig. 5 shows that during the initial 3 hours, when PG already reduced plasma P by 26 per cent (yet in previous studies the evolution of IUP was only fractional[1, 9–11]), cervical dilatation was less than 0.5 cm. Apparently DPGI (as PGI) promotes clinical progress only after several hours delay, when the reduced P levels permit *advanced* cyclic IUP, which approximates the ~200 mm. Hg maximum working capacity of the first- and 100 mm. Hg IUP of the second-trimester pregnant uterus ($P = 2\ wT/R$; where P = IUP, wT = wall tension, and R = radius). At 10 hours following DPGI, however, when Pw was ~37 per cent, cervical dilatation advanced to 2 cm. and clinical abortion was in advanced progress in the majority of patients.

Fig. 6 shows that, in contrast to intra-amniotic treatment, extraovular PG rapidly suspends the fetal heart sounds. This finding substantiates the premise

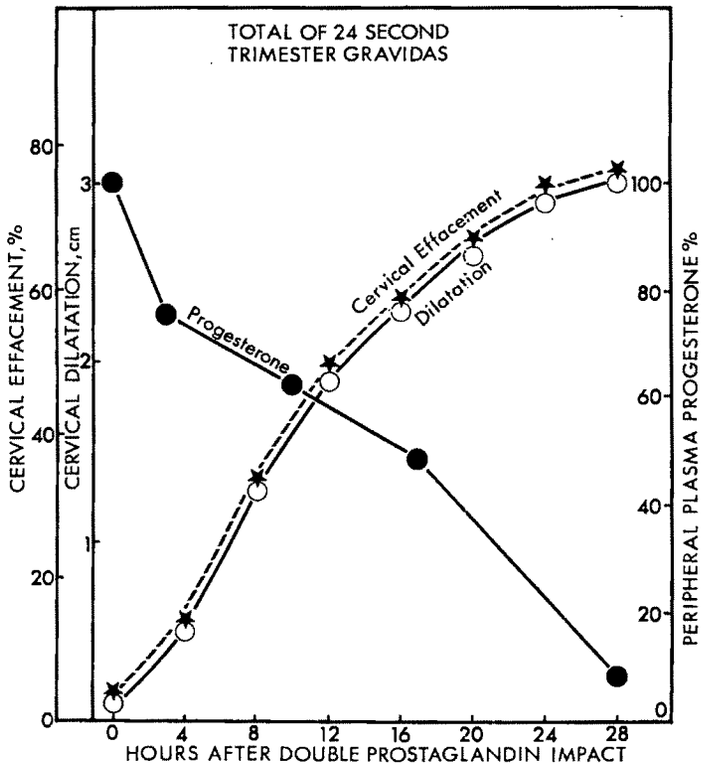


Fig. 5. The rates of Pw and cervical changes in second-trimester patients.

(to be discussed presently) that the effect of extraovular DPGI on the fetoplacental unit is more rapid and extensive than that of any other PG delivery system so far examined. As a result of failure in placental support, none of the fetuses was born alive, although seven patients were 18 to 20 weeks pregnant.

This premise—that DPGI rapidly suppresses fetoplacental function—is further substantiated by an additional observation (already established by earlier work[1, 4–9]) that effective PG treatment provokes rapid and drastic decrease in estradiol-17β (Fig. 6). This withdrawal was 54 per cent in 3 hours ($P < 0.001$), 68 per cent in 10 hours ($P < 0.001$), and 77 per cent at the time of abortion ($P < 0.001$).

Fig. 7 illustrates the cumulative abortion curve of the 100 study patients. Within 24 hours 86 per cent of the patients aborted. Apparently the clinical outcome of DPGI compares favorably with that of intra-amniotic PGI.[4]

The side effects and complications in this study were medically insignificant. Of the 100 sedated patients, 47 had mild, transient, and nonrecurring side effects. They occurred, as a rule, during or shortly after DPGI, in contrast to the early trials[3] when PG was administered through intravenous or extraovular infusions. Only in three gravidas was treatment discontinued because of severe vomiting. These three women aborted nevertheless, in spite of the low dosage received.

There were 31 incidents of nausea or vomiting, 16 hot flashes, 11 transient increases in blood pressure, seven occurrences of dyspnea, four of coughing, and three of diarrhea. Interestingly, in the second-trimester patients the severity and frequency of side effects were similar to those of the first-trimester gravidas, despite having received a double PGF2α dose. This finding suggests that the severity and frequency of (mild and transient) side effects are determined by the "sensitivity" of the patients to PG, or by the rate of initial adsorption, rather than by the total initial dose of PG. Of special interest is the negative finding that while the majority of patients recognized the initial contracture response of the uterus during DPGI, none of the 100 sedated volunteers showed symptoms of excessive pain during this exposure.

A total of 60 patients were curetted; however, in only 14 was curettage indicated by suspected placental residue. In the remaining 46 women, curettage was *diagnostic* and merely established that the uterine cavity was empty. A group of 40 patients were spared from curettage. Their postabortion spotting ceased 9.5 ± 0.5 days after DPGI (a period twice as long as those patients who were curetted). All study patients had spontaneous menstruation 30.6 ± 0.7 days after

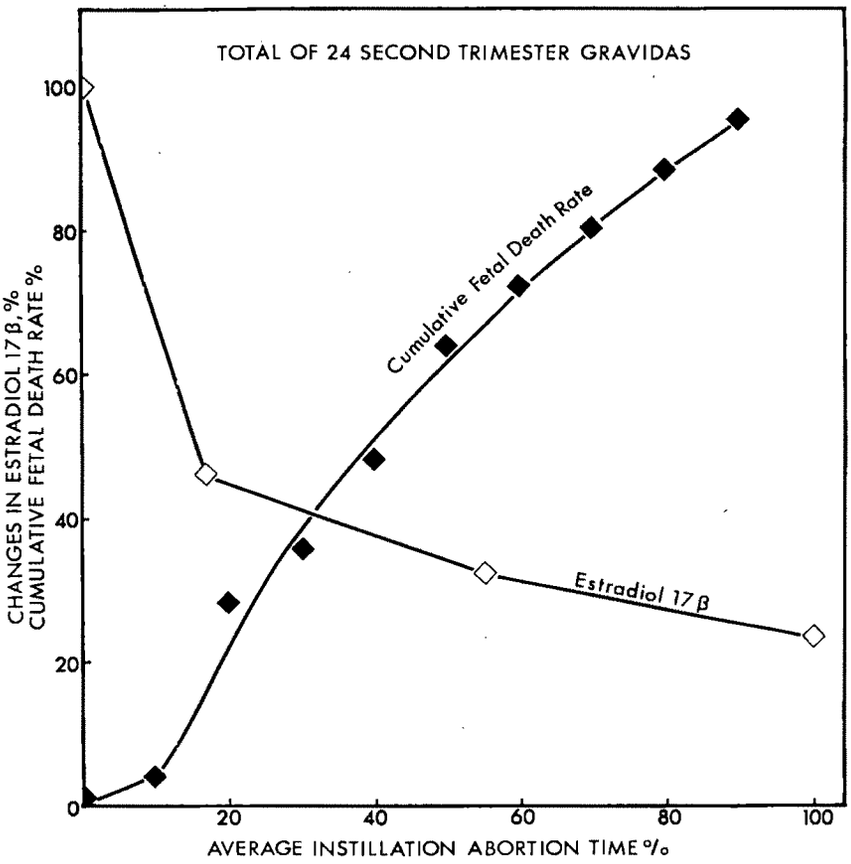


Fig. 6. The rates of E_{2w} and fetal death in second-trimester patients.

DPGI, which was normal in 82 per cent and less or more than normal in 18 per cent.

There were no complications, excepting eight cases of accidental rupture of the fetal membranes during catheter placement. These patients received intra-amniotic PGI and their IAT was prolonged. A total of nine cases of incomplete abortions and five residual postabortions demanded curettage. In spite of omitting curettage in 40 per cent of the study patients, there was only one case of transient elevated temperature. Of course, no gravida with cervicitis was admitted to the study group. The vagina was prepared with extreme care prior to DPGI, catheter placement was performed under strictly aseptic conditions, and after DPGI the treatment catheters were promptly removed. The patients were hospitalized for 1 to 2 days (depending on gestational age) and were repeatedly observed during this period. With the exception of one second-trimester patient, none of the remaining 99 women had elevated WBC or temperature beyond 38° C. during hospitalization, or reported fever after being discharged from the hospital. The one patient who had a peak temperature of 38.2° C. without elevated WBC had normal temperature after 2 days of antibiotic treatment.

Comment

The double prostaglandin impact (DPGI) technique successfully terminated 6 to 20 week pregnancies within 16.9 ± 0.9 hours in 99 out of 100 volunteers. Even the remaining one patient progressed to 2 cm. dilatation during 48 hours. In eight of the 99 gravidas accidental membrane rupture (during catheter placement) resulted in the intra-amniotic delivery of PGI. Of the 91 gravidas who received DPGI extraovularly, two thirds were treated only with the initial dose of 11.6 ± 0.5 mg. of PGF2α and aborted in 12.9 ± 0.7 hours, without requiring supplemental treatment. The remaining one third needed additional treatment, totalling 22.8 ± 1.6 mg. of PGF2α, and despite this increased dosage aborted in only 21.9 ± 2.1 hours.

This finding that at least in two thirds of the patients the theoretically "ideal" treatment has been closely approximated is informative, since there is no agreement among investigators regarding the most efficacious technique for the pharmacologic termination of

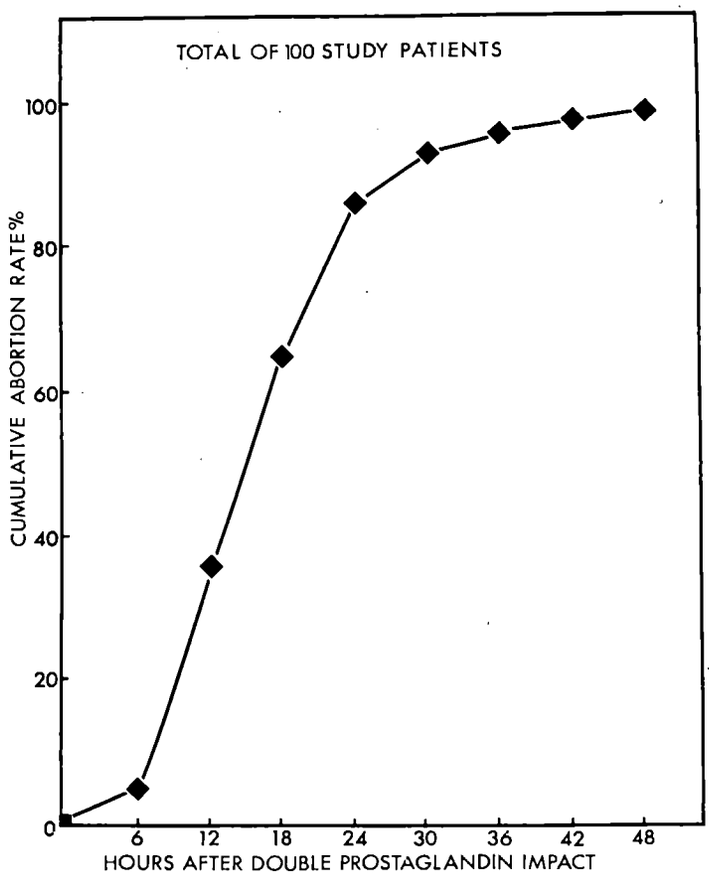


Fig. 7. The cumulative abortion rate of the 100 study patients.

pregnancy. If in the future repeated treatment and prolonged IAT could be avoided, DPGI (or a similar but further improved technique) might be considered for routine clinical use.

Thus the direction of future research is outlined. To promote its success, the question has to be raised: Why did the same DPGI technique provoke fast abortions in two thirds of the patients, without need for supplemental treatment, and slow abortions in one third of the patients despite supplemental treatment? It might be argued that this marked difference between the two groups was due to differences in the patients' "cervical conditions" and "sensitivity" to PG, since individual variations among patients undoubtedly affect the course of clinical progress. A distinct difference in the P levels of cases of treatment failures and successes and of fast and slow aborters has been documented already,[4–9] however, by over 25 reliable studies (Figs. 1 to 5). The sequential radioimmunoassays also established a close correlation between the rate and degree of Pw and the IAT. Regardless of how the patients were grouped (e.g., on the basis of parity, gestational age, initial or initial + supplemental treatment, total PG dose, etc. [see Figs. 1 to 4 and Tables III and IV]), the close correlation between Pw and IAT remained.

The evidence is, therefore, definitive that Pw does occur and that it precedes PG-induced abortion. Opponents of our contention that Pw plays a causal role in PG-induced abortion have to resort, therefore, to the argument that it is not Pw which provokes the evolution of IUP, but IUP provokes Pw. This argument rests on the premise that the *primary* action of the initial PG dose on the pregnant uterus is *oxytocic* and is exerted directly on the myometrial cells, rather than indirectly on their superimposed regulatory mechanism. If so, it can be further argued that it is this oxytocic action which, by generating high-level IUP and a consequent suppression of uteroplacental blood flow, provokes Pw.

The regulatory actions of PG and Pw on IUP being intimately related, the resolution of their temporal relationship (under the present study conditions, when both are changing) is difficult. It can be readily achieved when one of these regulators remains constant, however. Since the resolution of the sequence in which they affect IUP is a basic issue in reproductive

medicine, it is desirable to determine whether PGs induce the evolution of IUP after having provoked Pw or provoke this evolution regardless of P and thus Pw is a consequence of high-level IUP.

If shortly before or during early labor, when the cyclic IUP is only a fraction of the maximum working capacity of the term pregnant human uterus (~70 mm. Hg), a single dose of 25 m.U. of oxytocin or 25 μg of PGF2α is administered to patients in a slow intravenous injection, the myometrium promptly displays ~70 mm. Hg cyclic IUP.[10] This effect (frequently used clinically to determine the "inducability" of patients) is the *classic oxytocic response* of the uterus. The maximum uterine response (at different stages of gestation) is predicted by the Laplace theorem (IUP = 2 wT/R), which states that when uterine wall tension (wT) is constant, IUP is inversely related to the uterine radius (R). Calculation, as well as experimental evidence, show[1, 4–10] that when the reactive uterus is maximally activated, the IUP is controlled by R and thus the second-trimester uterus develops over 100 mm. Hg and the first-trimester uterus about 200 mm. Hg IUP. This demonstration, of course, is possible only if the recording technique of IUP is accurate (e.g., the recording system is not leaky), the fetal membranes are intact and not bulging, there is no partial block in the recording system, and observations continue until the descent of the uterine contents.[10]

Thus, if PGs could exert on the human myometrium a primary oxytocic action during the first half of gestation (the basic premise of the argument opposing our contention) then within 5 minutes PGs should provoke 100 to 200 mm. Hg *cyclic* IUP (depending on R). No one ever observed such a uterine response. Only an unphysiologic *contracture* has been recorded, on which fractional cyclic IUP was superimposed, increasing *gradually* with the progress of *time*.[1, 4–10]

Evidentially, as stated in the "see-saw theory,"[5] PGs cannot *exert* during normal pregnancy a classic oxytocic response on the *refractory* human uterus until it is *converted* into a *reactive* organ by a regulatory imbalance. Thus the premise that PGs provoke high-level IUP through a primary oxytocic action and Pw through that effect on IUP stands unsupported. For, initially PGs do not provoke maximal cyclic IUP; indeed, as late as 3 hours after treatment (when Pw is already highly significant, $P < 0.001$, Table III), the cyclic IUP is still only a fraction of the maximum working capacity of the organ.[1, 4–10]

Since, however, after PG treatment, Pw and the evolution of cyclic IUP follow a similar time course, it is desirable to demonstrate that IUP is controlled by P, rather than the other way around. This demonstration demands a "clean system," in which only P is changing while PG is constant. Accordingly, Pw was provoked in early pregnant women by luteectomy (rather than by PG) and the oxytocic response of the uterus was examined daily to standard intravenous doses of 250 m.U. of oxytocin and 250 μg of PGF2α.[11] It then became apparent that until P decreased to a *critical* value (below 5 ng. per milliliter in cases of early pregnancy), the myometrium *only* responded to constant doses of these powerful oxytocics with transient contractures. High-level cyclic IUP did not appear until P decreased to the critical value. Evidentially, as predicted on the basis of extensive earlier studies,[5] the "sensitivity" of the pregnant human uterus to oxytocics is controlled by P, and thus Pw must *precede* the evolution of IUP. This study also demonstrated that luteectomy provokes abortion by Pw (rather than through an increase in endogenous PGF2α). Even the unphysiologically high dose of exogenous PGF2α (250 μg intravenously) only provoked *contracture*, rather than advanced cyclic IUP, until P decreased to the critical value. As documented by the present (Tables III and IV) and earlier results,[4–11] this critical P value is higher during late first and second trimesters than during early pregnancy.

If Pw precedes the evolution of IUP and oxytocic response, the question is, how do PGs provoke Pw? There is no evidence that in the human subject PGs directly interfere with P genesis, hence the inefficacy of PGs during the luteal phase of the menstrual cycle. In contrast, there is evidence, in both animal "models"[12] and patients,[13] that PGF2α *promptly* provokes *vasoconstriction* in the uteroplacental vessels long before advanced cyclic IUP develops and suppresses uteroplacental blood flow. The prompt and excessive contracture response of the pregnant uterus to PGs is most probably an expression of this circulatory effect of PGs (anoxia).

Jointly, vasoconstriction and contracture can effectively suppress fetoplacental endocrine function and cause continued Pw. Once provoked, Pw reduces the excitability *threshold* of the myometrium[14] and *converts* the refractory uterus to a reactive organ. When converted, the myometrium responds to both endogenous and low-level exogenous PG stimulation.[5] The fact[1, 4–10] that maximal cyclic IUP evolves only several hours after the administration of a single dose of PGF2α (when this exogenous compound is already metabolized), rather than shortly after treatment (when PG concentration is the highest), further supports the contention that PGI is therapeutically effective because it provokes Pw (rather than an oxytocic action). PG stimulation after Pw is therapeuti-

cally desirable, however, since its effect is additive to that of endogenous PGs. Recent measurements of placental P[15] indicate that PG-induced Pw might be more drastic and rapid than is reflected by the circulating plasma P levels. In any case, once effective Pw did provoke the evolution of advanced cyclic IUP, the promotion of activity becomes *autocatalytic,* in that increased IUP further promotes activity through increased reduction of uteroplacental blood flow and fetoplacental endocrine function.

This reconstruction of the mechanism of PGI is, of course, tentative and precise documentation of the sequence of events requires further investigation. From a practical point of view, however, this initial reconstruction has been useful already in explaining why sustained PG infusions and repeated medium doses (given with the intent of direct myometrial stimulation) were clinically ineffective, in spite of excessive and medically unacceptable side effects.[3] Reconstruction also provided a readily measurable endpoint for PG therapy: effective Pw. Rapid radioimmunoassays can inform physicians in a few hours whether PG treatment has been successful. This information could be specifically valuable in cases of "menstrual induction,"[16] when the distinct clinical signs of effective therapy do not evolve as rapidly as they do in cases of more advanced pregnancy.

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The Frontier Nursing Service: The primary care nurse in the community hospital

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On the occasion of the Fiftieth Anniversary of the Frontier Nursing Service, we extend our congratulations for the excellent health care system the Frontier Nursing Service has developed and provided in a medically underserved area, for the progress made, and for the attainment of professional and community cooperation.

The Editors

THE NURSE as a primary care provider was first introduced into rural southeastern Kentucky fifty years ago by the Frontier Nursing Service (FNS). The aim was to establish a viable health care system in medically underserved areas. The nurse-midwife was selected as the most appropriately trained health care provider to meet the problems of the rural areas. She was more easily attainable at less cost than the physician. She could manage the common day-to-day health problems encountered by the family and, most important, take care of the mother throughout the childbearing period and her newborn infant. She was prepared in health teaching and in public health.

The patients with more complex problems requiring medical care were sent to physicians and hospitals in neighboring areas until a hospital could be built and a physician added to the staff. Through this system, the family was never more than one hour's travel time from primary care; now, with improved transportation, it is one half hour. Through established links with State health agencies, University Medical Centers, and other specialty care clinics in the State, the most sophisticated care could be made available to the most isolated family.

In 1970, the nurse-midwifery training program, established in 1939, was broadened to include family nursing, by the addition of diagnosis and management of common health problems. This preparation markedly extends the skills of the nurse. It enables her to perform selected medically delegated tasks in combination with her nursing skills—the caring, teaching, counseling, and management skills. The combination of these skills helps to make her care meaningful to the family, and the close working relationship with the physician and other members of the health team helps to assure the family of quality care. The physician at all times retains ultimate responsibility for medical services and is available around the clock to work with the nurse in management of more complex problems.

The nurse in all areas, hospital, clinic, and home, works with Medical Directives—medical protocol mutually developed by medicine and nursing. These "directives" specify privileges and limitations of practice and provide guidelines for the nurse in the management of common health problems, including prescription of therapeutic measures and drugs and designation of the point at which consultation must be sought or the patient must be referred to the physician. For effective utilization of these directives, the nurse needs to be prepared in history taking, physical examination, common laboratory procedures, diagnosis of disease, health assessment, and the management of common health problems.

Hospital inpatients

The role of the primary care nurse in the FNS hospital may vary substantially. In most situations, the

From the Frontier Nursing Service.

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patient requiring hospitilization is referred by the nurse to the physician who makes the final determination. The nurse may hospitalize the patient in the absence of the physician. This privilege is utilized primarily for situations in which the patient needs nursing observation and care for such considerations as croup or minor injuries, or conditions not considered serious enough to require the immediate attention of the physician but which the family cannot manage at home. In all situations, the physician must be notified within 12 hours after admission. Policies defining the privileges and limitations for hospital admission by the nurse have been established and are filed with the Kentucky Hospital Association.

Primary care nursing functions vary within the hospital, depending on the type of patient served. The family nurse is responsible for all admission physical examinations on the general ward and the pediatric nurse on the pediatric ward. In the process, she is expected to obtain a social and family history, if this has not been done previously. This provides a base for planning care, counseling, health teaching, and discharge. The family is involved as appropriate in the care of the patient. In the process, the family is taught health care of the patient and receives assistance in the management of their own life-style in relation to the needs of the patient. Admission physical examinations are reviewed by the physician, who sees the patient twice daily and as indicated. The nurse continues to follow patient care management, performing nursing and medically delegated functions as indicated. The nurse may write routine admission orders, in accordance with the Medical Directives and hospital policies, but consults with the physician for special orders.

All patient care plans are reviewed daily by the physician-nurse team. Other members of the health team may be included. The amount of teaching and counseling provided by the nurse is dependent on the needs of the patient. Discharge summaries are written by the physician. The family nurse, the pediatric nurse, and the physician consult frequently with each other and the staff nurses (registered nurses and/or licensed practical nurses assigned to the ward) to assure continuity of care and for teaching purposes. Monthly Hospital Utilization Reviews are attended by representatives from the total team, with the aims of improving patient care and establishing appropriate standards.

On the obstetric ward, the nurse-midwife is responsible for the management of all normal obstetric patients that have had prenatal care, and the normal newborn infant. The obstetrician is called when a patient is admitted who has not had prenatal care or when abnormalities which may complicate labor and/or delivery are noted. A pediatrician is called if any abnormalities arise in relation to the fetus or newborn infant. In case of complications, the nurse-midwives and physician work together as a team in the management of patient care. Over 90 per cent of the babies are delivered by the nurse-midwife. Nurse-midwives may perform episiotomies and repairs and use the vacuum extractor. Physicians perform cesarean sections and forceps deliveries when indicated. The nurse-midwife is specially trained for care in the normal situation, so when an abnormal situation occurs, she is quick to recognize it and seek the assistance of the physician. Special attention is paid to the high-risk mother and her infant.

The Metropolitan Life Study of the FNS Midwifery

Table I. Leading causes of maternal deaths: FNS and national*

Cause	*FNS (1925 to 1954, per 10,000 deliveries)*	*United States (1939 to 1941, per 10,000 live births)*
Sepsis	2 (1 Laceration of cervix) (1 With cesarean section)	9.1
Toxemia	1 (1 With cardiac complications)	8.7
Abortion	—	6.8
Hemorrhage	3 (3 Post partum)	4.6
Ectopic pregnancy	—	1.5
Other	5 (2 With pneumonia) (2 With cardiac complications) (1 Pulmonary edema)	5.5
Total	11	36.3

*Source of national statistics: Annual Volumes of Vital Statistics of the United States, National Center for Health Statistics, United States Public Health Service, Washington, D. C.

Table II. Comparative rates of premature births, stillbirths, and neonatal deaths per 1,000 live births.

	*FNS (1952 to 1954)**	*United States (1953)†*
Premature births	37.6	76.0
Stillbirths	9.2	17.8
Neonatal deaths	17.3 (7 term, 10 premature)	19.6

*Source of FNS statistics: Report on the Tenth Thousand Confinements and the First Thousand Confinements of the Frontier Nursing Service, Inc., by Metropolitan Life Insurance Company, 1958.

†Source of national statistics: Annual Volumes of Vital Statistics of the United States, National Center for Health Statistics, United States Public Health Service, Washington, D. C.

Table III. Twenty-year summary of FNS Midwifery Service statistics compared with Kentucky rates (May, 1954 to April, 1974)

Years	No. of live births (FNS)	Stillbirths			Maternal deaths		Neonatal deaths		
		No. (FNS)	Rate*		No. (FNS)	Rate‡ (State†)	No. (FNS)	Rate*	
			FNS	State†				FNS	State†
1954-1959	2,405	32	13.3	15.1	0	4.0	42	17.4	20.0
1959-1964	1,987	24	12.0	14.4	0	4.4	40	20.1	19.1
1964-1969	1,613	16	9.9	13.5	0	2.1	20	12.3	16.9
1969-1974	1,259	17	13.5	12.6	0	2.3	16	12.7	13.9
Total	7,264	89	12.2	14.1	0	2.8	118	14.8	17.8

The source of information for the state statistics was *Kentucky Vital Statistics,* Department of Health, Frankfort, Kentucky. N.A. = Not available.

*Rate per 1,000 live births.

†Midpoint year used for state ratios and percentages.

‡Rates per 10,000 live births.

§During the 1969 to 1974 period, increased stillbirths and neonatal death rates were accompanied by a rubella and influenza epidemic in the area served.

Service of the first 10,000 deliveries between 1925 and 1954 indicates 11 maternal deaths (two were not attributed to obstetric causes) as compared with a national maternal mortality rate of 36.3 per 10,000 live births for the midpoint period, 1939 to 1941. Table I illustrates the causes of maternal deaths, national and in the FNS.

The low maternal death rate is particularly significant since 60 per cent of deliveries between 1925 and 1954 were conducted in the home in an extremely poverty-stricken area, where the main mode of transportation was by horseback, modern facilities and medical assistance were difficult to attain, and the percentage of high-risk mothers and infants was great.

The numbers of premature births, stillbirths, and neonatal deaths deliveries performed by the FNS were also lower than the national averages, as indicated in Table II. Comparable figures are available for 1952 to 1954 only.

In all situations where neonatal or maternal deaths occurred, complications were noted during the prenatal period. The study demonstrates that early recognition and management of complications in pregnancy can have a major impact on the mortality rate of high-risk mothers and infants in rural areas.

Since 1952, statistics indicate no maternal deaths in over 8,000 deliveries and a continuing decline in the stillbirth and neonatal mortality rates among 7,264 babies delivered by the FNS between May, 1954, and April, 1974, as noted in Table III. However, the decline is not as rapid as that noted in the Kentucky rates. The birth rate in the area served reached a peak of 41 per 1,000 population in 1965. This subsided rapidly with the introduction of a family planning program which is provided through the nurse-midwifery program with medical support.[2] In 1973, the provisional birth rate for the area served was 17.8 per 1,000 population.

Ambulatory care

The primary care nurse in the ambulatory care clinics at the FNS may be best described as the front line worker. In the fiscal year 1974, approximately 60 per cent of the ambulatory visits were made by primary care nurses located in eight nursing satellite clinics. Forty per cent of these patients were seen in the home, and the remainder were seen in the nursing clinics. Thirty-four per cent of the patients seen were 15 years of age or less; 21 per cent were 60 years of age or over, and 14 per cent were midwifery patients. These form the groups of patients most commonly neglected in our present-day health care system.

Most of the patients seen were in the "no abnormality" category: the prenatal, postpartum, and family planning patients; the newborn, well-child, and school health groups; and patients who desire immunization, a Papanicolaou smear, breast examination, tuberculosis testing, and routine physicals for health care, education, and eating establishment employees.

The most common disorders seen are upper respiratory infections, otitis media, hypertension, arteriosclerotic heart disease, influenza and bronchitis, kidney and urinary tract infection, diabetes mellitus, and chronic obstructive pulmonary disease, in that order. Common infectious disease and minor disorders are managed according to medical protocol. Patients

Deliveries attended by physician			*Home deliveries*		
	Percentage			*Percentage*	
No. (FNS)	*FNS*	*State†*	*No. (FNS)*	*FNS*	*State†*
53	2.2	N.A.	996	41.4	N.A.
32	1.1	97.6	559	28.1	4.7
71	4.9	98.4	134	8.3	2.1
99	7.3	99.1	28	2.2	0.9
255	3.5	97.3	1,717	23.6	4.0

with chronic disease are identified by the nurse for referral, and follow-up visits are made after a diagnostic work-up by the physician with a plan of care. Medical protocol is specifically developed for these purposes. If the response to treatment is unsatisfactory, medical consultation is sought.

Radio and telephone communications with the physician and other members of the health team are available at all times. The more complex conditions not readily managed with medical protocol are referred to the hospital, physician, or social worker as indicated. A physician visits each district once or twice a month on a regular schedule.

Weekly utilization review meetings are held by the home health care team, including the physician, the district nurse supervisor, the social worker, and the district nurse. Patients on medical care plans are reviewed and other health problems that arise are brought up for discussion. The aims are to assure appropriate health manpower utilization, quality, and high standards of care and to provide a teaching mechanism.

At the hospital clinic, the nurse obtains all initial histories and performs all physical examinations, and she may order specified laboratory procedures or x-rays as indicated. Medical consultation or referral is sought as indicated. Medical Directives provide the basis upon which the nurse initiates care. A physician is on call around the clock and sees all patients requiring the skilled care of a physician. During the day, the physician is available in the hospital; after 4 P.M., he is on call.

A modified problem-oriented record system is used and monitored by the physicians. This helps assure appropriate utilization of family nursing and medical skills and a high standard of health services. It also serves as an excellent teaching tool.

The primary care nurse helps to combine preventive measures with the management of common health problems. Heavy emphasis is placed on helping families to cope with their own health problem. The aim is to lessen the need for costly hospitalization and nursing home care. Only 10 per cent of patients admitted to the hospital are 65 years old and over, which is equivalent to their distribution in the population group. Their average length of stay in the hospital is 7.6 days, which is approximately half of the average length of stay in similar community hospitals. Most chronically ill patients are referred to home health. Only the rare patient is referred to a nursing or personal care home.

The savings for the State and Federal government and the patient created by the primary care system developed by the FNS have not been figured, and a suitable method for measurement of the quality of care has not been found. Research in this area is underway.

Perhaps one of the most important aspects of this type of health care program is the manner in which it helps strengthen or maintain family ties and helps individual members assume greater responsibility for one another. In times of childbirth or illness, there is a natural pulling together of the family. If various members within the family are supported in their efforts to help one another and the necessary guidance is provided, much can be done to alleviate the stress and loneliness of illness and to increase the joy and satisfaction of parenthood. If the health care system is organized in a manner whereby primary care is readily accessible with good specialty care linkages, much can be done to lessen chronic disability and perhaps maintain and revive the community spirit that used to be so vital in the development of this country.

The primary care nurse, whether prepared in family nursing, pediatrics, or midwifery, can do much to broaden the dimensions of health services available and make health care more meaningful to our citizens, particularly in rural and other medically underserved areas. But she cannot do this in isolation or without the whole hearted support of the physician and the other members of the health team.

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Intravascular coagulation in obstetrics: Serial dilution protamine sulfate test throughout labor

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Serial dilution protamine sulfate tests (SDPS) were performed in 191 women during labor. It was found that 27 per cent of women with uncomplicated pregnancies and 42 per cent with complicated pregnancies had positive SDPS tests, the highest incidence being between three and six hours of labor. These findings support the previous report of a physiologic disseminated intravascular coagulation in pregnancy but make the SDPS test of little value in obstetric cases.

THE EXISTENCE OF a process of physiologic intravascular coagulation during normal labor has been postulated based on the rise of fibrinogen degradation products and on the significant fall in plasma fibrinogen and factor VIII.[1] In 1970, Kleiner and associates[1] suggested that the mechanism involved is qualitatively similar in both normal and pathologic conditions, but it is of higher magnitude in the latter as in cases of abruptio placentae or intrauterine death.

In the present study we have tried to use the induction of the paracoagulation phenomenon with the use of serial dilution protamine sulfate test (SDPS) described by Niewiaroski, Gurewich, and Hutchinson[2, 3] as a means of identifying disseminated intravascular coagulation (DIC) in women undergoing labor.

Material and methods

Patients studied. Blood specimens were tested from 191 women chosen at random in the delivery room.

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Table I

	Normal labor		*Complicated labor*	
Parity	*No. of cases*	*SDPS (per cent positive test)*	*No. of cases*	*SDPS (per cent positive test)*
0-1	69	32*	29	34*
1-11	33	40*	14	64*
11 Or more	18	11*	28	32†

*$p < 0.01$ for each pair of series.

†$p > 0.70$ for the difference between primigravida and multiparous patients.

Labor starting time and associated pathology were recorded. Single blood samples were drawn from 171 women, and in 60 serial samples were obtained at two-hour intervals during labor. Seventy-one patients experienced some sort of complication during the process (pre-eclampsia, intrauterine death, cephalopelvic desproportion, premature rupture of membranes, etc). All women were followed for 24 hours after delivery.

Citrated plasma. Blood, 4.5 ml., was drawn in disposable plastic syringes and mixed in polystyrene tubes with 0.5 ml. of sodium citrate (3.2 per cent) which contained either ten per cent epsilon amino caproic acid (EACA) or 2000 U. of aprotinin. The blood was centrifuged immediately at 3,000 r.p.m. for ten minutes. Plasma was separated with the use of plastic

Table II

Diagnosis	*No. of cases*	*SDPS (No. of positive cases)*	*SDPS (per cent positive)*
Premature rupture of membranes	18	7	38.8
Cephalopelvic desproportion	17	6	35.2
Pre-eclampsia	17	9	52.9
Intrauterine death	7	3	42.8
Miscellaneous	12	5	41.6
Total	71	30	42.0
Normal	120	32	27.0

Table III. Protamine sulfate test samples according to hours of labor

	Hours of labor				
	0-3	*4-6*	*7-9*	*10-12*	*13-15*
Total samples	46	89	71	50	13
Positive test (per cent of samples)					
Normal labor	20	27	18	16	11
Abnormal labor	31	38	19	17	0

pipettes and was tested the same day or kept frozen at −20° C. until used.

Protamine sulfate (Roche), one ampule, was opened daily and dilutions of 1/5, 1/10, 1/20, 1/40, and 1/80 were made in tris-saline buffer, 0.05M, pH 6.5; 0.2 ml. of each dilution was added to 0.2 ml. of plasma, mixed, and left for 30 minutes at room temperature. Reading was done with the naked eye with the use of a fluorescent lamp against a black background. The results were expressed as coarse precipitate, fibrin strands, and gel. All samples which showed either fibrin strands or gel in one or several dilutions were considered positive.

Results

There were no differences between samples taken either with EACA or aprotinin or between the samples tested fresh or frozen.

In normal labor the incidence of positive SDPS in primigravid and secundigravid women was 32 and 40 per cent, respectively, compared with 11 per cent in multiparous patients ($p < 0.01$). In complicated labor the incidence was 34 per cent for primigravid women, 64 per cent for secundigravid women and ($p < 0.01$) 32 per cent for multiparous women ($p < 0.70$) (Table I). Of these patients only ten with normal pregnancies and two with complicated pregnancies had previous histories of miscarriage, and only one of each group had positive SDPS test.

In the group from whom serial samples were taken every two hours, it was found that in seven of the women with normal pregnancies (eight per cent) and nine of those with complicated pregnancies (22 per cent) in whom the tests were negative at the beginning of labor, the tests became positive with the following samples.

Table II shows the incidence of positive SDPS tests according to the diagnosis. Although the number of subjects tested is rather small in each pathology, the percentage of positive cases is higher than that in normal subjects ($p < 0.001$).

Table III shows the results of SDPS tests in uncomplicated and complicated pregnancies during labor. Samples were grouped in periods of three hours according to the onset of labor. The positive tests were expressed as percentage of samples in each period. In normal labor an increase of positivity during the first six hours is shown, declining thereafter during the subsequent periods. In abnormal labor the highest percentage of positive tests was also observed during the first six hours, but the number of positive cases was about 50 per cent higher than that in women with normal labor. No hemorrhagic complications were observed in any of the SDPS-positive patients.

Comment

Pregnancy seems to be accompanied by DIC which is greater in those patients who tend to become complicated with pre-eclampsia and other factors which increase the prenatal morbidity and mortality rate.[4–8]

Hyde and co-workers,[9] with the use of the SDPS test to detect the presence of thrombin-induced fibrinogen derivatives in plasma of women during pregnancy and puerperium, have found a significantly increased incidence of positive SDPS tests in uncomplicated primigravid pregnancies compared with secundigravid and multiparous pregnancies and an influence on the positivity of a history of previous miscarriages. Our findings in normal and complicated labor are similar,

but the highest positivity occurred in secundiparous patients and is not affected by the history of miscarriage (Table I).

Hyde and associates[9] also found an incidence as high as 47.5 and 43.6 per cent of positive SDPS tests in the second and third trimesters of patients who had normal pregnancies and 68.5 per cent in pre-eclamptic patients. Therefore, we could expect a similar incidence during labor; but surprisingly the highest percentage of positivity we found was 27 per cent for normal and 38 per cent for complicated labor during the first six hours, with a total incidence of 27 per cent and 42 per cent, respectively (Tables II and III). These values are much lower than those of the above-mentioned authors.

The work performed by the myometrium during labor increases from a starting pressure of 25 mm. Hg up to a pressure of 100 mm. Hg during fetal expulsion.[9] It might be assumed that as labor progresses the increase of uterine activity could result in a progressive release of thromboplastin from the placental tissue,[10] the activation of the hemostatic mechanism becoming greater with a higher incidence of SDPS. Nevertheless, our results do not confirm this hypothesis, since in both the normal and abnormal series the highest incidence was found in the first six hours of labor with a significant decrease after seven hours (Table III). At the moment we do not have an explanation in each case. Further studies are underway in order to elucidate this problem.

In conclusion the lack of correlation between the positive results and the absence of hemorrhagic complications makes the SDPS test of limited value to detect DIC in obstetric patients.

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CP 24,270

Amount and distribution pattern of soluble fibrin monomer complexes during the early puerperium

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Soluble fibrin monomer complexes (SFMC) were determined in 12 patients following delivery, 2, 4, 6 days and 3 months post partum. Quantitative gel filtration (4 per cent agarose) of the β-alanine–precipitated plasma samples yielded the relative (per cent of the total fibrinogen content) and absolute (milligrams per 100 ml. of plasma) amount of SFMC. During the early puerperium the amount of SFMC remained essentially constant, with average postpartum values of 6.3 ± 1.2 per cent and 27.6 ± 9.1 mg. per 100 ml. (mean and standard deviation). Three months after delivery the level of SFMC was significantly ($p < 0.001$) decreased (3.3 ± 1.3 per cent and 8.4 ± 3.4 mg. per 100 ml.). The quantitative estimation of SFMC in the early puerperium as presented in this study indicates that a state of hypercoagulability can be evaluated by measuring the thrombin-mediated catabolic products of fibrinogen.

THE OCCURRENCE of soluble fibrin monomer complexes (SFMC) during delivery is a rather frequent phenomenon which might be related to the so-called state of hypercoagulability during gestation. Recently a fibrinogen derivative with a higher molecular weight than that of the parent molecule was observed in plasma samples from normal pregnant patients.[1, 2] The physicochemical and immunologic properties of this component are in agreement with the assumption of a dimeric structure which may occur either as a fibrinogen fibrin monomer complex or as a covalently linked dimer.[2] It is eluted during gel filtration on 4 per cent agarose gel in a shoulder prior to the fibrinogen peak.

The frequency of venous thrombosis and pulmonary embolism is significantly increased during the puerperium. Observations during this period may give further information concerning the occurrence of SFMC and their relation to hypercoagulability and thromboembolism. Quantitative gel filtration was therefore applied in the present study to determine the amount and distribution pattern of SFMC during the early puerperium.

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Material and methods

Collection of blood specimens. Twelve healthy primiparas and multiparas, 23 to 39 years of age, were examined. Pregnancy, delivery, and postpartum course were in all cases uneventful. All newborn infants had an Apgar score of 8 to 10.

Blood was obtained within the first 12 hours of the immediate postpartum period, on day 2, 4, and 6 of the puerperium, as well as 3 months after delivery. The blood samples were drawn from the cubital vein under constant mixing with the anticoagulant (1:10) (trisodium citrate 3.8 Gm., TES 1.38 Gm., 10 ml. of Trasylol, Bayer, Leverkusen, Germany, to 100 ml. at pH 7.5). Platelet-poor plasma was prepared by centrifugation in a refrigerated centrifuge. When it was necessary to store samples, they were held at −25° C. up to 4 weeks.

The fibrinogen content of plasma was assayed by measuring the optical density (280 to 325 nm.) of the clottable protein[3] from 0.5 ml. of plasma (dissolved in 10 ml. of 40 per cent urea, 0.2N NaOH, and 1 ml.

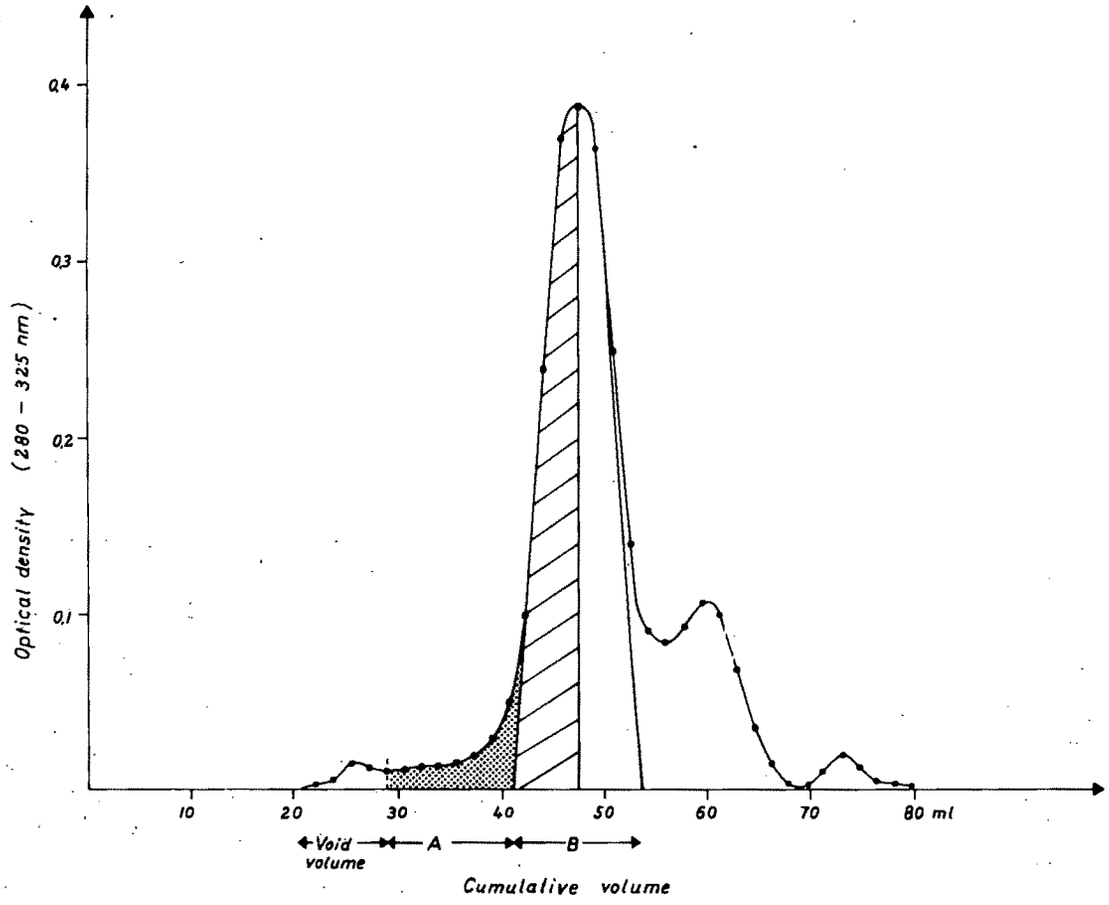


Fig. 1. Elution pattern following agarose gel filtration (Biogel A-15 m) of a β-alanine precipitated plasma sample during the early puerperium. Optical density (280-325 nm.) shown by the dotted curve. The cut of the eluate between the elution position of the void volume and the ascending part of the fibrinogen peak *(A)* is compared with the one of the fibrinogen peak *(B)*. The percentage of SFMC (in relation to the total fibrinogen content) is determined according to the formula: SFMC per cent = A × 100/A + B.

0.3M NaCl) using an extinction coefficient of fibrin in alkali as 15.87 ($E^{1\%}_{1cm}$).[4] The measurements were performed in duplicate and the evaluation of the precision of the assay method yielded a coefficient of variation attributable to the method of 2.8 per cent.

Purification procedure. Fibrinogen and its derivatives were precipitated from plasma with β-alanine. Crystalline β-alanine was added at 0° C. to the plasma specimen (2.5 ml.) up to a final concentration of 2.5M and the precipitate was redissolved in Tris-Cl-citrate buffer (pH 7.6) containing 100 KIU Trasylol per milliliter.[5]

Quantitative agarose gel filtration. A 4 per cent agarose gel filtration was performed with Biogel A-15 m spherical agarose, 100 to 200 mesh (Bio-Rad Laboratories, Munich, Germany). A 1 ml. sample containing 5 to 8 mg of protein was applied to a 1 by 90 cm. column and eluted with Tris-Cl-buffer, pH 7.6. The flow rate was 10 ml. per hour (1.6 ml. per fraction). Double determinations were performed by using two columns in parallel. The optical density of the fractions was measured at 280 and 325 nm. with a Hitachi 181 spectrophotometer. The elution position of the void volume of each column was determined by use of Dextran Blue and that of the fibrinogen peak by use of commercially available human fibrinogen (FFBH, Deutsche Kabi GmbH, Munich, Germany).

In earlier studies it was demonstrated that the presence of SFMC in plasma is evidenced by the elution of fibrinogen-related antigen (FR-antigen) material in a shoulder prior to the fibrinogen peak and that the material eluted in this range between the elution position of the void volume and the ascending part of the fibrinogen peak consists mainly of a component with the relative electrophoretic mobility of 0.28×10^{-5} cm.²/V × sec. in polyacrylamide gel.[2, 6] This cut of the eluate was planimetrically evaluated and compared with the one of the fibrinogen peak

Table I. Amount of soluble fibrin monomer complexes (SFMC) and of fibrinogen in plasma during the early puerperium and 3 months after delivery (mean ± standard deviation)

	Postpartum				*t test*	
	<12 hr. after delivery I	*2 days II*	*4 days III*	*6 days IV*	*a: I vs. V b: I-IV vs. V c: III vs. IV*	*3 mo. after delivery V*
SFMC (per cent of total fibrinogen content)	6.1 ± 1.5	6.2 ± 1.8	7.0 ± 2.0	6.0 ± 1.9	a: $p < 0.001$ b: $p < 0.001$ c: $p > 0.05$	3.3 ± 1.3
SFMC (mg. per 100 ml.)	27.4 ± 10.5	28.1 ± 12.0	29.1 ± 11.6	21.8 ± 9.8	a: $p < 0.001$ b: $p < 0.001$ c: $p < 0.05$	8.4 ± 3.4
Fibrinogen (mg. per 100 ml.)	445 ± 139	453 ± 131	404 ± 111	369 ± 97	a: $p < 0.001$ b: $p < 0.005$ c: $p > 0.1$	269 ± 99

(weight of the respective paper cut areas) (see Fig. 1). The percentage of SFMC in relation to the total fibrinogen content was determined according to the formula SFMC per cent = A × 100/A + B, where A is the area of the shoulder and B the one of the fibrinogen peak. The presence of FR-antigen material in the eluted fractions of the shoulder and of the peak was confirmed in certain cases by use of the staphylococcal clumping test (RKW 10, Behringwerke A. G., Marburg, Germany). The precision of the assay method was evaluated by double determinations. The coefficient of variation attributable to the method was 8.7 per cent. The absolute amount of SFMC in milligrams per 100 ml. of plasma was calculated by relating the relative amount of SFMC to the fibrinogen concentration in the respective plasma sample.

Statistical evaluation. Distribution of the data was evaluated by use of a probit type plot. Mean values and standard deviations were calculated and Student's t test for paired observations was utilized to compare the data from the early puerperium with those from 3 months after delivery. The Wilcoxon test for paired observations was additionally applied.

Results

The results of the determinations of SFMC and fibrinogen are summarized in Table I. During the immediate postpartum period and until day 6 of the puerperium the relative (per cent of total fibrinogen content) as well as the absolute (milligrams per 100 ml.) amount of SFMC remained essentially constant. The mean values of the relative amounts of SFMC varied between 6.0 and 7.0 per cent, the ones of the absolute amounts between 21 and 29 mg. per 100 ml. (mean and standard deviation of the average postpartum values collected from all patients, 6.3 ± 1.2 per cent; 27.6 ± 9.1 mg. per 100 ml.).

No statistically significant differences were observed until day 4. Between days 4 and 6 a statistically significant decline was observed for the absolute amount of SFMC (milligrams per 100 ml.) ($p < 0.05$) only.

The level of SFMC was considerably lower 3 months after delivery than in the early puerperium and was estimated at 3.3 ± 1.3 per cent of total fibrinogen content and at 8.4 ± 3.4 mg. per 100 ml. (mean ± S.D.). The differences between the values from the immediate postpartum period and those from 3 months after delivery (a) as well as the differences between the average postpartum values of each patient (b) and those from 3 months after delivery were statistically significant on a 0.001 level.

The fibrinogen content of plasma during the immediate postpartum period until day 6 of the puerperium remained at levels between 369 ± 97 and 445 ± 139 mg. per 100 ml. (mean ± S.D.). No statistically significant differences were observed in this collection period. Yet the values 3 months after delivery were considerably lower (269 ± 99 mg. per 100 ml., mean ± S.D.), and the difference between the immediate postpartum period and 3 months after delivery was statistically significant at the 0.001 level of probability (average of all postpartum values vs. values at 3 months after delivery: $p < 0.005$).

Though the optical impression of the probit type plot from the data of the first collection period indicated a normal distribution pattern, a nonparametric test was additionally applied. The Wilcoxon test for paired observations confirmed the results described above.

Comment

The data presented indicate that increased levels of SFMC are observed during the early puerperium as compared to 3 months post partum. Further identification of the material which was eluted in the shoulder

prior to the fibrinogen peak was not possible in this study because of the small amounts of protein eluted. Yet the similarity of the elution pattern after 4 per cent agarose gel filtration of the β-alanine–precipitated plasma samples with the one in earlier studies[2, 6] strongly suggests that the fractions from the shoulder contain predominantly a component with a dimeric structure which may occur either as a fibrinogen fibrin monomer complex or as a covalently linked dimer, and which has a relative electrophoretic mobility in 5 per cent polyacrylamide gel of 0.28×10^{-5} cm.^{2}/V × sec. ("0.28 derivative"). A fibrinogen derivative with the elution behaviour and the electrophoretic mobility of the 0.28 derivative was also observed after in vitro addition of small amounts of thrombin to plasma.[6, 7] The striking similarity between the in vitro findings and the observations in plasma samples from patients suggests that the 0.28 derivative is also in vivo induced by the effect of thrombin or of a thrombinlike enzyme on fibrinogen.

The "hypercoagulable" state of the puerperium with its increased incidence of thromboembolic episodes is thus well reflected by an elevated level of SFMC in vivo. It might be assumed that the SFMC described in this study is related to the cryoprofibrin characterized by Shainoff and Page.[8] Sherman[9] recently reported a considerably shorter half-life time for ^{125}I-cryoprofibrin in rabbits (13 hours) than for fibrinogen. If similar biological t ½ values in human subjects are assumed, the unaltered increased level of these products till day 4 of the puerperium would indicate that an increased formation of soluble fibrin is maintained over this period. This increased fibrinogen catabolism is additionally reflected by the elevated fibrinogen level in plasma. The slight, though statistically significant, decline of the absolute amount of SFMC (milligrams per 100 ml.) from day 4 to day 6 may signal the return of the increased fibrinogen catabolism to normal.

The planimetric evaluation of the gel filtration pattern tends, according to the procedure involved, to result in probably too high estimations, if the shoulder prior to the fibrinogen peak is very small. Therefore, the indicated level of approximately 3 per cent of the total fibrinogen content for a normal female 3 months after delivery could well be overestimated. Yet in this regard, it seems of interest to note that Hörmann and Ströder[10] already demonstrated oligomers of fibrin in a cold insoluble fraction from normal human plasma. An over-all coefficient of variation applicable to the method of 8.7 per cent seems to warrant a sufficient precision of the assay.

The presence of SFMC in plasma samples of pregnant or postpartum patients was also demonstrated by other techniques such as cryoprecipitation,[11, 12] serial dilution protamine precipitation,[11] or by ethanol gelation,[2, 13, 14] but a more direct and quantitative approach will probably lead to more definite conclusions.

The quantitative estimation of SFMC in the early puerperium as presented in this study indicates that a state of hypercoagulability can be evaluated by measuring the in vivo occurring, thrombin-mediated catabolic products of fibrinogen. This may be of help for the early diagnosis of venous thromboembolic disease. In addition, this evaluation of hypercoagulability may be of diagnostic value in cases of intrauterine growth retardation, as suggested by Frigoletto and his colleagues,[14] or of pre-eclampsia.[11] Together with the qualitative identification of high molecular weight derivatives of fibrinogen in plasma[15] it may also lead to a more detailed description of the degree of disseminated intravascular coagulation in a given case.

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Indomethacin induces rat uterine contractions in vitro and alters reactivity to calcium and acetylcholine

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The initial contractions of uteri in vitro from castrated, estrogen-treated rats, were markedly diminished following replacement with fresh bathing medium. Indomethacin and aspirin (10^{-5} to 10^{-4}M) strongly stimulated such quiescent preparations and reduced their subsequent responsiveness to Ca^{++}. Reintroducing the initial bathing medium (which contained prostaglandin-like material), or adding prostaglandin $F_{2\alpha}$ to the fresh medium, initiated uterine contractions and restored responsiveness to calcium ion. Injections of indomethacin into castrated, estrogen-treated rats reduced initial in vitro uterine motility, abolished production of prostaglandin-like compounds, and prevented either indomethacin, aspirin, or Ca^{++} from stimulating uterine contractions. Uterine responsiveness to acetylcholine in vitro was significantly reduced in rats pretreated with indomethacin.

PROSTAGLANDINS are now thought to be major intrinsic regulators of uterine contractility.[1] Prostaglandin production may increase following activation of the uterus by estradiol,[2] and prostaglandin $F_{2\alpha}$ levels are dependent on the stage of the menstrual cycle in the human Fallopian tube.[3] Two established inhibitors of prostaglandin synthesis, aspirin and indomethacin,[4] have been reported to abolish uterine contractions in vitro.[5, 6] They also prolong parturition in the rat[7] and the monkey,[8] presumably by blocking endometrial prostaglandin synthesis. Salicylates, however, elicit an apparently contradictory result—premature parturition in mice.[9] Informal reports suggest that aspirin also may precipitate abortion in women during early pregnancy.[10]

Prostaglandin production often contributes to spontaneous contractions that smooth muscles develop in vitro. Adding fresh bathing medium may remove these prostaglandins, abolish or reduce initial motility, and alter the response of a smooth muscle preparation to calcium ion and other stimulatory agents.[11] Indomethacin affects not only prostaglandin synthesis but also calcium balance.[12] We, therefore, investigated the effect of changing the Tyrode's solution prior to administering indomethacin, aspirin, Ca^{++}, or prostaglandin $F_{2\alpha}$ to rat uterine preparations in vitro. We also studied the effects of indomethacin injections prior to sacrifice on rat uterine contractility, prostaglandin production, and responsiveness to acetylcholine.

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Materials and methods

Two groups of six ovariectomized female rats received 10 μg per kilogram of estradiol benzoate in 0.2 c.c. of corn oil daily for 5 days. One of the groups also received 10 mg. per kilogram of indomethacin in corn oil intraperitoneally during the final 2 days and 1 hour prior to sacrifice. The uteri were excised and suspended in all-glass, water-jacketed muscle warmers containing 20 ml. of oxygenated Tyrode's solution (37° C.) immediately after the rats had been killed by decapitation. Contractions were recorded isometrically against a tension that was equivalent to less than 0.5 g. The uteri were incubated for 30 minutes to allow recovery from thermal or operative shock, and fresh

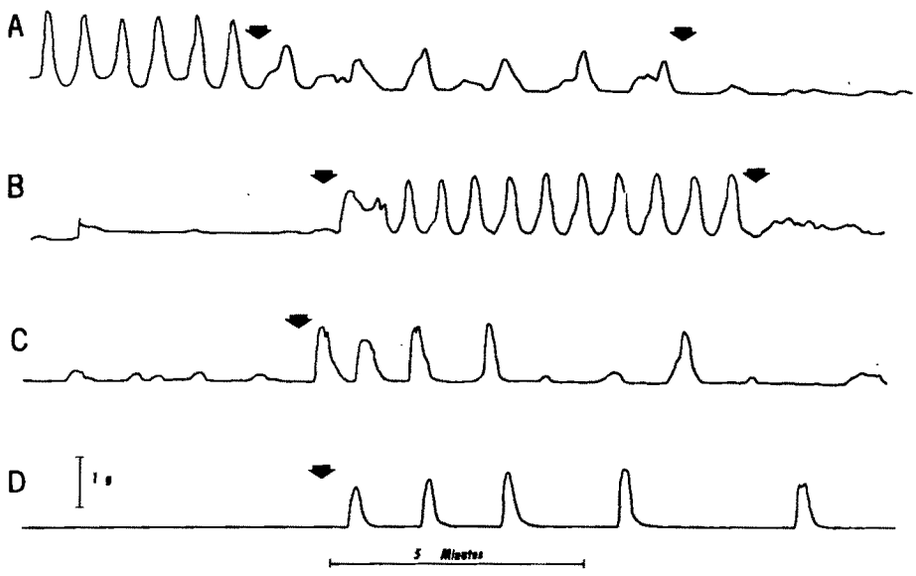


Fig. 1. *A*, Reduction of rat uterine contractility in vitro following two changes of the bathing medium (indicated by arrows). *B*, Restoration of contractility after initial medium from an active preparation was added back to quiescent preparation (first arrow), with reduction in activity after one rinse (second arrow). *C*, Contractions induced by addition (at arrow) of 28 μM indomethacin, followed by tachyphylaxis. *D*, Contractions in response to 0.10 mM. aspirin (arrow) followed by tachyphylaxis. Vertical bar is equivalent to a pen deflection caused by 1 g; preparation volume equals 20 ml.

medium was added prior to treatment of inactive preparations with pharmacologic agents. Indomethacin, aspirin, and $PGF_{2\alpha}$ were dissolved in ethyl alcohol and added to the bathing media in aliquots of 5 to 100 μl. $CaCl_2$ (5 mM.) was dissolved in 20 ml. of Tyrode's solution and put directly into the muscle warmer.

Two additional groups of four rats were similarly ovariectomized and treated with estradiol (10 μg per day for 5 days); one such group received indomethacin (10 mg. per kilogram) on the final 2 days and 1 hour before sacrifice. Uteri from these animals were suspended in Tyrode's solution as stated above and used to generate log dose-response curves in response to acetylcholine (Sigma).

Prostaglandin extraction and chromatography

Initial bathing media (20 ml. volumes) were collected following the 30 minute incubation period and stored at −20° C. Media from four preparations of each treatment group (estradiol alone, or indomethacin plus estradiol) were pooled, acidified to pH 3.0 with HCl, and partitioned three times against equal volumes of distilled ethyl acetate. The organic solvent was collected and dried at 40° C. in rotary evaporators. Residues were taken up with three 5 ml. volumes of ethyl acetate, transferred to 15 ml. capacity test tubes, and evaporated under nitrogen gas. Lipidic material was dissolved in 50 μl of ethanol and spotted on chromatographic plates coated with a slurry (0.25 mm. thick) of 30 Gm. of silica gel H (Merck) and 6 Gm. of silver nitrate. Plates containing authentic prostaglandin were developed with phosphomolybdic acid for confirmation of the R_f values reported by Green and Samuelsson.[13] The silica gel coat was scraped from the plates on which the extracts had been spotted in zones corresponding to these R_f values, transferred to centrifuge tubes, and eluted three times with 5 ml. aliquots of methanol to recover any prostaglandin-like material. This solvent was then evaporated under nitrogen gas at 40° C. To remove salts from the residue, the material was redissolved in 5 ml. portions of phosphate buffer (pH 3.0) and partitioned three times against equal volumes of ethyl acetate. After evaporating the organic solvent, the residues were taken up in Tyrode's solution for bioassay on the rabbit testicular capsule as reported.[14] To estimate the per cent recovery of prostaglandins, 0.5 μg of PGE_1 and 1.0 μg of $PGF_{2\alpha}$ were added to Tyrode's solution; this fluid was extracted and chromatographed in the same manner as the uterine bath fluid prior to bioassay.

Results

Uteri from estrogen-treated rats contracted autorhythmically in vitro at a greater amplitude than those treated with estrogen and indomethacin (Table I). The contractions were markedly diminished or

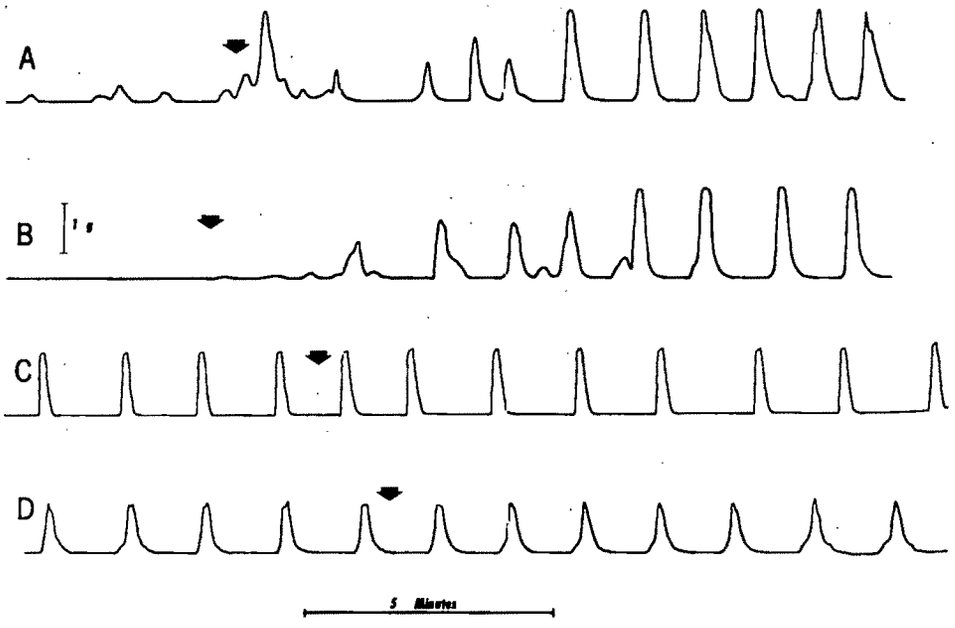


Fig. 2. *A*, Stimulation of inactive rat uterus by 0.14 mM. indomethacin (added at arrow). *B*, Stimulation of inactive rat uterus by 0.28 mM. aspirin (added at arrow). *C*, Lack of inhibition of rat uterine contractility following addition (at arrow) of 0.035 mM. indomethacin. *D*, Lack of inhibition of actively contracting rat uterus after addition (at arrow) of 0.056 mM. aspirin.

totally abolished by a change of the bathing medium, but were restored if the original medium was reintroduced (Fig. 1, *A* and *B*). Extraction of the original media for acidic lipids followed by chromatography and bioassay demonstrated that media from estrogen-treated preparations contained prostaglandin-like material in zones corresponding to authentic prostaglandins $F_{2\alpha}$, $F_{1\alpha}$, and E_1, but not E_2. However, indomethacin-pretreated preparations were less motile than untreated units, and produced no detectable stimulatory material (Table I).

The contractile responses of quiescent preparations to aspirin or indomethacin proved to be dose-dependent, with a brief stimulation followed by tachyphylaxis seen at 10^{-5} to 10^{-4}M final concentrations (Fig. 1, *C* and *D*). At concentrations greater than 10^{-4}M, both aspirin and indomethacin initiated contractions in previously inactive preparations (Fig. 2, *A* and *B*); this motility persisted for at least 30 minutes in vitro. Replacing the media with fresh Tyrode's solution abolished this contractility (data not shown). However, when actively contracting uteri were treated with doses of these compounds from 3.5×10^{-5} to 1.4×10^{-4}M, neither marked stimulation nor inhibition was observed (Fig. 2, *C* and *D*). Inactive preparations from indomethacin-pretreated animals responded to neither aspirin nor indomethacin with contractions (Table II).

Uteri from estradiol-pretreated rats responded to Ca^{++} with increased contractility; this phenomenon was particularly evident in quiescent, rinsed preparations (Fig. 3, *A*). Uteri from indomethacin-pretreated rats, however, reacted little to the addition of Ca^{++} to the medium (Fig. 3, *B*; Table II). In every case 7×10^{-7}M $PGF_{2\alpha}$ completely restored uterine responsiveness to Ca^{++} (Fig. 3, *C*). Ethanol (10 to 100 μl) did not markedly affect either spontaneously active or inactive, rinsed preparations (Fig. 3, *D* and *E*).

Table I. Initial amplitude of contraction and prostaglandin-like material recovered from the bathing media of uteri from estradiol-treated (10 μg per day) and estradiol plus indomethacin-treated (10 mg. per kilogram) rats.

	Estradiol ($n = 6$)	*Estradiol and indomethacin* ($n = 6$)	*P*
Initial contractions (g displacement)	1.65 ± 0.37	0.98 ± 0.31	<0.05
Prostaglandin-like material* (ng. per 20 ml. bath fluid)			
$PGF_{2\alpha}$	320	0	—
$PGF_{1\alpha}$	150	0	—
PFE_2	0	0	—
PGE_1	30	0	—

*Corrected for recovery of 78 per cent.

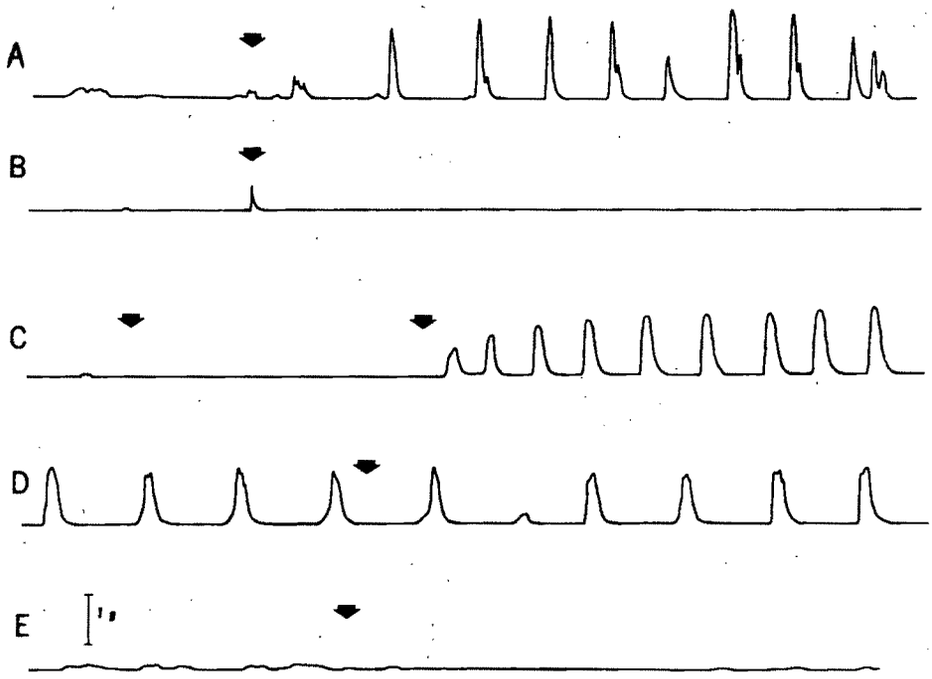


Fig. 3. *A*, Stimulation of rat uterine contractility by 7 mM. $CaCl_2$ (added at arrow). *B*, Lack of effect of 7 mM. $CaCl_2$ (arrow) on quiescent uterus from indomethacin-pretreated rat. *C*, Lack of effect of 7 mM. $CaCl_2$ (first arrow) followed by contractions induced by addition of 71 nM. prostaglandin $F_{2\alpha}$ (second arrow). *D*, Slight inhibition of actively contracting rat uterus caused by addition of 50 λ of ethanol (42 mM. final concentration) at arrow. *E*, Lack of stimulation of quiescent rat uterus by 50 λ of ethanol added at arrow.

Pretreatment of spayed female rats with indomethacin (108 mg. per kilogram) prior to sacrifice significantly reduced the responsiveness of uterine preparations to acetylcholine ($p < 0.05$; Fig. 4, *A* and *C*) when compared to the response of uteri from control animals. Addition of prostaglandin $F_{2\alpha}$ to the media at threshold levels (7×10^{-9}M) failed to increase sigficantly the response to acetylcholine of uteri from animals pretreated with indomethacin ($p > 0.20$; Fig. 4, *B*).

Comment

Nonsteroidal, anti-inflammatory drugs may inhibit smooth muscle contractility. Northover[12] has attributed this effect to an alteration by indomethacin of Ca^{++} binding by subcellular membranes. Other workers have found prostaglandin output to decrease following indomethacin or aspirin treatment of rat uterine and other preparations.[5, 6] In addition, cyclic AMP production increases in rat gastric mucosa in the presence of aspirin.[15] We here report an additional, stimulatory effect of these drugs on smooth muscle contractility, in consonance with aspirin's ability to bring about premature parturition in mice.[9] One plausible cause of increased motility would be a displacement of Ca^{++} from subcellular membranes, leading to temporarily high levels of unbound, intracellular Ca^{++}. Reduced motility would be expected following an efflux of this ion from smooth muscle cells. Our observations that uteri from indomethacin-pretreated rats are initially quiescent in vitro, and that these uteri respond to either acetylcholine or Ca^{++} at a low level, accord with such a train of events.

In a previous report, Vane and Williams[6] did not find any change in uterine sensitivity to acetylcholine after indomethacin treatment in vitro. These workers, however, added the indomethacin directly to the uterine bathing medium for brief periods (30 to 90 seconds), whereas we injected rats with this compound intraperitoneally for 2 days prior to the experiment. In addition, their failure to observe increased contractility could be due to their use of preparations that were already motile as a consequence of prostaglandins present in the bathing medium. By first rinsing the uteri with fresh Tyrode's solution, we reduced contractility, thereby increasing the sensitivity of the preparation to aspirin or indomethacin. Our present results support the conclusion drawn from previous studies[5, 6] concerning the contribution of prostaglandins to smooth muscle motility in vitro, since uteri from rats

Table II. Contraction frequency (10 minute interval) of uteri from estradiol-treated (10 μg per day) rats vs. those from estradiol plus indomethacin-treated (10 mg. per kilogram) rats in response to stimulation by indomethacin, aspirin, and $CaCl_2$

	Estradiol ($n = 6$)	*Estradiol and indomethacin* ($n = 6$)	*P*
Aspirin (2.8×10^{-4}M)*	10.67 ± 0.88	0.5 ± 0.21	<0.001
Indomethacin (1.4×10^{-4} M)*	8.75 ± 0.63	0.0	<0.001
$CaCl_2$ (7 mM.)	9.2 ± 0.80	0.83 ± 0.41	<0.001
$CaCl_2$ (7 mM.) + $PGF_{2\alpha}$ (5×10^{-7}M)	14.1 ± 0.53	12.0 ± 0.23	>0.20

*0.05 mg. per milliliter.

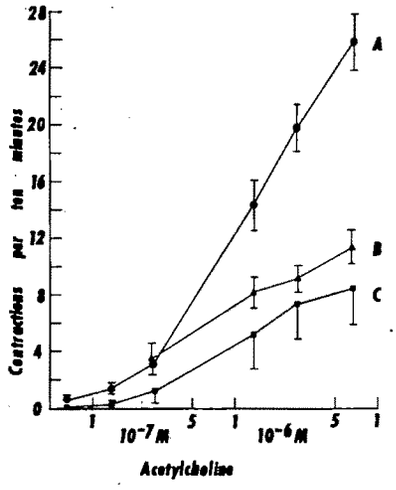


Fig. 4. *A*, Log dose-response (LDR) curve for the acetylcholine-caused contraction rate in uteri from estradiol-pretreated rats. *B*, LDR curve for acetylcholine stimulation of uteri from estradiol- and indomethacin-pretreated rats, in the presence of 7 nM. prostaglandin $F_{2\alpha}$. *C*, LDR curve depicting the response of uteri from estradiol- and indomethacin-pretreated rats to acetylcholine. Vertical bars equal ± the standard error of the mean.

pretreated with indomethacin produced no prostaglandin-like material and were inactive. In addition, prostaglandin $F_{2\alpha}$ always restored uterine contractions and responsiveness to Ca^{++}. As did Vane and Williams,[6] we failed to detect any prostaglandin E_2 in the media from actively contracting preparations, although other PG's were present. We found no direct inhibition of active uteri by either aspirin or indomethacin, however. We conclude, therefore, that reports of immediately reduced motility may be attributable to rinsing the preparations, thereby removing any prostaglandins that may already have been present. Alternatively, indomethacin or aspirin may directly inhibit uterine contractions, regardless of the presence of prostaglandins, at the lower concentrations used by other researchers.[5, 6]

The evidence presented here suggests that, in addition to exerting inhibitory effects on prostaglandin synthesis, indomethacin and aspirin directly affect smooth muscle. Observed activation of uterine musculature by high doses of anti-inflammatory compounds may explain the premature induction of labor by indomethacin or aspirin,[9, 10] whereas their reported delay of parturition[7, 8] may be due to their effects on prostaglandins, cyclic AMP, or Ca^{++} balance. It now seems apparent that the actions of indomethacin and aspirin may not be totally explained by their inhibiting prostaglandin synthesis.

We thank Dr. John E. Pike of the Upjohn Co., Kalamazoo, Michigan, for the prostaglandins, and Dr. Horace Brown of Merck, Sharp and Dohme, Rahway, New Jersey, for the indomethacin used in these studies.

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Prognostic value of antenatal screening

JAMES WALKER, M.D.

Dundee, Scotland

Rates of stillbirths and first-week deaths have fallen steadily in Scotland in the last 30 years. Seventy-five per cent of all such deaths in the City of Dundee now follow the clinical condition of "antepartum hemorrhage," "premature labor," "poor fetal growth," and "lethal deformity," all of which are conditions associated with poorer environmental conditions which are worse in larger industrial cities. Therefore, we are concerned with these clinical situations and with fetal death and subsequent morbidity of children born in those situations. This paper describes in summary a planned study of antenatal screening with assessment of fetal placental hormones, fetal biparietal diameter, and other determinants of fetal well-being, the growth of the child and weight at birth, the quality of the labor, the neonatal state, and the ultimate quality of the child.

CERTAINLY, SINCE 1940, there has been in Scotland a steady and continuous fall in the perinatal mortality rate (stillbirths after 28 weeks plus first-week deaths).

The reasons for this fall are a steady improvement in the health of the community (although nearly 25 per cent of women of reproductive age are still less than 5 feet, 1 inch in height) some change in the age and parity distribution, and, of course, greatly improved obstetric and early neonatal care.

Value of antenatal screening

In the City of Dundee, there was a high birth rate in 1960 to 1963 because more women were having third, fourth, or fifth babies. The birth rate has now fallen to 12.3 per 1,000 in 1973, because of fewer women having more than two children. There is a fall in the number of first pregnancies possibly because of some postponement of child-bearing, but 35 per cent of all births are now to primigravidas and 30 per cent are second pregnancies — only 7 per cent are fifth or later pregnancies. Forty-five per cent of our mothers are under 25 years of age, and only 4.7 per cent are over 35.

However, it is possible to evalute the *clinical* situation in which improvement has occurred only when each individual death is classified by the clinical cause responsible for the death.[1]

From the Department of Obstetrics and Gynecology, University of Dundee.

Presented by invitation at the Ninety-eighth Annual Meeting of the American Gynecological Society, Coronado, California, April 9-12, 1975.

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Table I. Dundee perinatal mortality rates per 1,000 single legitimate pregnancies

Years	*DEF*	*APH*	*PU*	*MD*	*TOX*	*TR*	*MU*	*O*
1956-1959	8.8	5.7	5.3	2.8	2.5	3.0	3.6	1.1
1960-1963	9.3	4.7	5.3	1.7	1.4	1.7	3.1	1.9
1964-1968	6.1	3.4	3.8	1.6	0.7	1.1	2.4	1.3
1969-1973	5.1	2.5	3.0	1.0	0.2	1.2	1.5	1.3
		10.6			5.0			
1974	8.2	0.9	5.0	0.45	0.9	0.9	1.4	1.8
		72%						

DEF = Lethal deformity; APH = antepartum hemorrhage; PU = premature unknown; MD = maternal disease; TOX = toxemia; TR = trauma; MU = mature unknown; O = other.

Such a classification has been applied to national death on a limited scale, but intensive analysis of perinatal deaths in the cities of Aberdeen, Dundee, and, more recently, Glasgow has been undertaken by the technique. In Dundee, it will be seen that, since 1956, there has been a steady fall in death rates in all clinical cause groups (Table I). Provisional figures for 1974 show that 72 per cent of all perinatal deaths are in the clinical cause group of "lethal deformity," "antepartum hemorrhage," and "premature unknown" and that 25 per cent of all deaths are in fetuses weighing less than 1,000 grams.

Comparison of clinical death causes and rates in infants of unselected Glasgow women with those of "selected" Dundee married women having a single pregnancy (Table II) show that the major difference between the two is in the same groups, i.e., the

Table II. Perinatal mortality rates per 1,000 in Glasgow, 1970, and in Dundee, 1969-1973

City	*DEF*	*APH*	*PU*	*MD*	*TOX*	*TR*	*MU*	*Other*	*?*
Glasgow (27.6)	6.3	5.6	6.0	0.7	1.7	1.4	3.0	1.3	1.6
Dundee (15.6)	5.1	2.5	3.0	1.0	0.2	1.0	1.5	1.3	

For explanation of abbreviations, see footnote to Table I.

"environmental" group plus the "mature unknown" group which is often associated with late pregnancy failure. Causes easily amenable to obstetric care are roughly equal in the two cities.

Perinatal death of the mature infant is no longer a serious clinical problem, and we are now concerned almost entirely with the environmental problems of lethal deformity, premature labor, and retarded intrauterine growth and with death resulting from these causes or with the "quality" of the child surviving these situations. Therefore, we must study all these events of pregnancy and labor which might influence physical and mental growth and development of the fetus or which may physically damage its mechanisms or its normal state.

Labor and the events of labor as producers of risk are covered by intensive monitoring systems. However, perinatal death associated with labor and the immediate postnatal period has a rate (corrected for deformity) of some 3 per 1,000, and it is clear that intensive monitoring of labor will save the lives of very few babies indeed. However, it may minimize morbidity. The intelligent concern of the obstetrician is, as we have said, now centered more on the "quality of the child produced" and on those events of pregnancy and labor which may influence that quality. There is much evidence that the fetus in a poor growth situation is, of course, particularly at risk of long-term handicap from added labor risk.

It has become customary to talk of the "at-risk pregnancy" selected on clinical grounds for special antenatal and labor care. Multiple antenatal and labor monitoring systems are in the process of evolution and evaluation largely at random in the somewhat frantic search for guidelines to the well-being of the individual fetus and as a measure of the stress to which he has been subjected. In view of this, it was decided, in 1974, by the Departments of Obstetrics and Gynaecology, Clinical Chemistry, and Paediatrics, of the University of Dundee, that a systematic study should be developed of the clinical value of the various biochemical, physical, and physiologic tests available to us.

Three groups of patients were selected: (1) those with a bad obstetric history, especially of pregnancy failure; (2) a mixed group of primigravidas which were divided into a group likely to provide normal pregnancy (tall, well-nourished, and 20 to 30 years of age) and a group in which poor fetal growth and prematurity were likely (low social class, small, and under 25 or over 35 years of age).

The screening study

Antenatal assessments. Urine samples (24 hours) were analyzed for "total" estrogen content at 30, 34, and 38 weeks as a check against other findings. Blood samples taken after 16 weeks regularly throughout pregnancy and during labor for estimations (mostly by radioimmunoassay techniques) of human chorionic gonadotropin, human placental lactogen, estriol, estradiol, progesterone, and alpha fetoprotein (others in special cases). The biparietal diameter of the fetal head was determined by ultrasound at similar stages in all pregnancies.

During labor. Maternal blood samples, electronic monitoring of the fetal heart rate, and intrauterine pressure were recorded, and the scalp blood pH was measured if necessary. Maternal uterine vein samples were taken where the abdomen was open.

At birth. Umbilical vessel samples were taken for hemoglobin and haematocrit, blood gas, and blood hormone estimations as in the mother. Babies were then automatically specially followed up by the neonatal pediatric service. A long-term follow-up is being undertaken by the pediatric assessment organization already mounted within the city.

To date, we have screened approximately 150 mothers. Their babies have also had unbilical vein samples and are now in pediatric follow-up. About a further 100 will be delivered in the next six months. We propose to cease the screening by late summer of 1975, and extensive analyses will be made.

Results

Sample preliminary results of three analyses are presented.

Alpha fetoprotein. Alpha fetoprotein was demonstrated in serum from the human fetus in 1956,[2] and, in 1967, its source was noted to be the fetal liver in the human subject.[8] (The possibility of some production by the human yolk sac was not fully investigated; in the rat, the yolk sac is a common source.)

It was shown[4] that in anencephalic pregnancy the

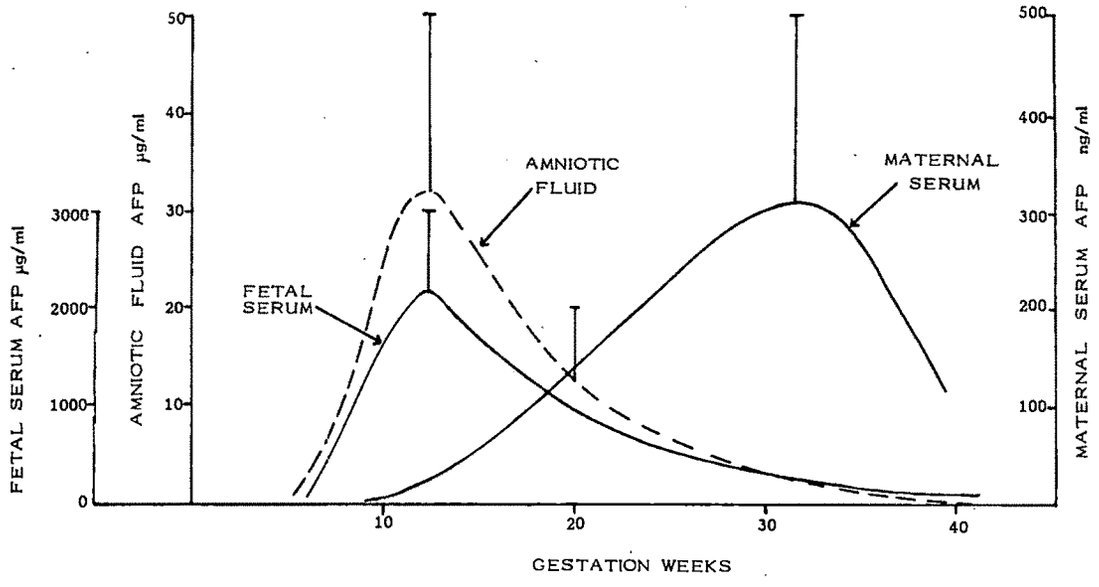


Fig. 1. Alpha fetoprotein *(AFP)* in amniotic fluid and in fetal and maternal serum.

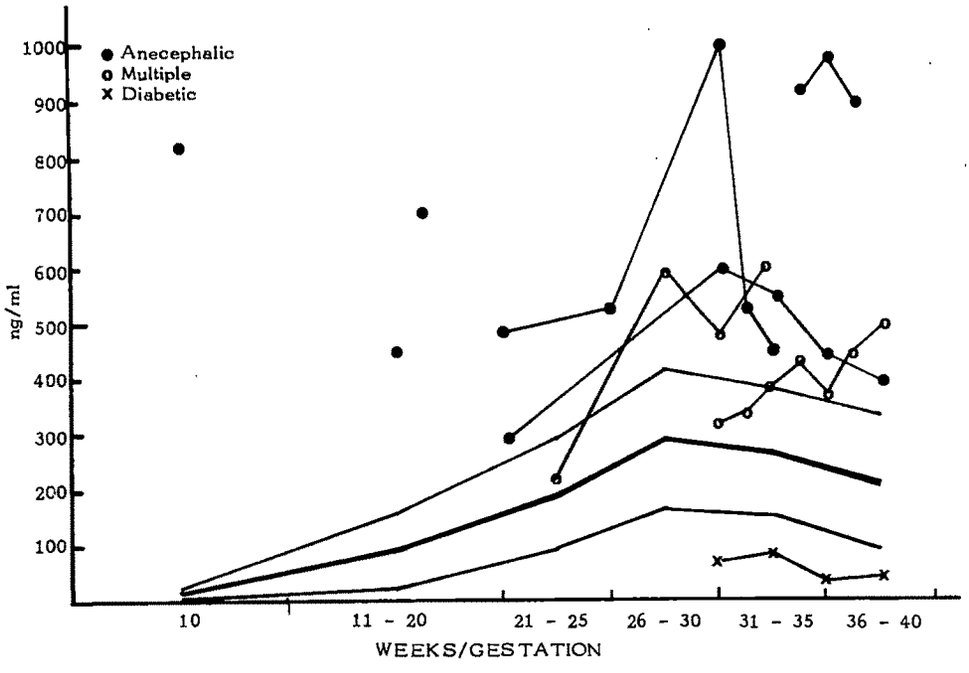


Fig. 2. Alpha fetoprotein in maternal serum—normal pregnancy (mean ± one standard deviation), pregnancy with anencephaly, and multiple pregnancy.

alpha-fetoprotein concentration in the amniotic fluid was well above the normal range before 25 weeks of gestation but that the difference from the normal range was not quite so clearly defined after that time. Brock and Sutcliffe[4] were the first to suggest that alpha fetoprotein could act as a *marker molecule* to select pregnancies where termination would prevent the birth of an anencephalic child or a child severly affected by spina bifida.

Subsequent evidence suggests that high readings in the amniotic fluid are seen only in the serious degrees of spina bifida and not in minor degrees, especially in closed spina bifida (15 per cent of the total) which will respond to postdelivery therapy. High readings in

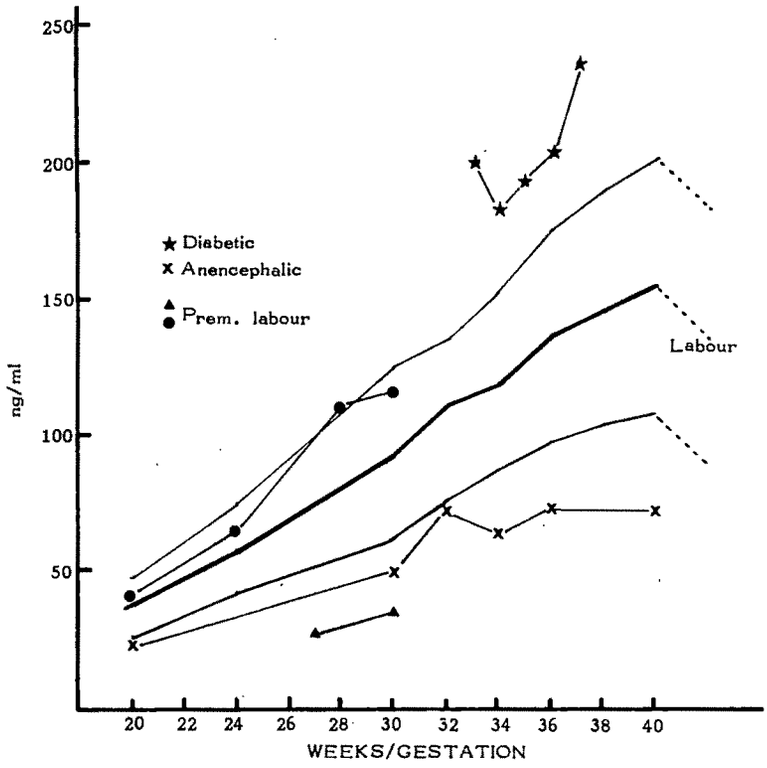


Fig. 3. Progesterone in maternal plasma (mean ± one standard deviation) in normal pregnancy and in some abnormal conditions.

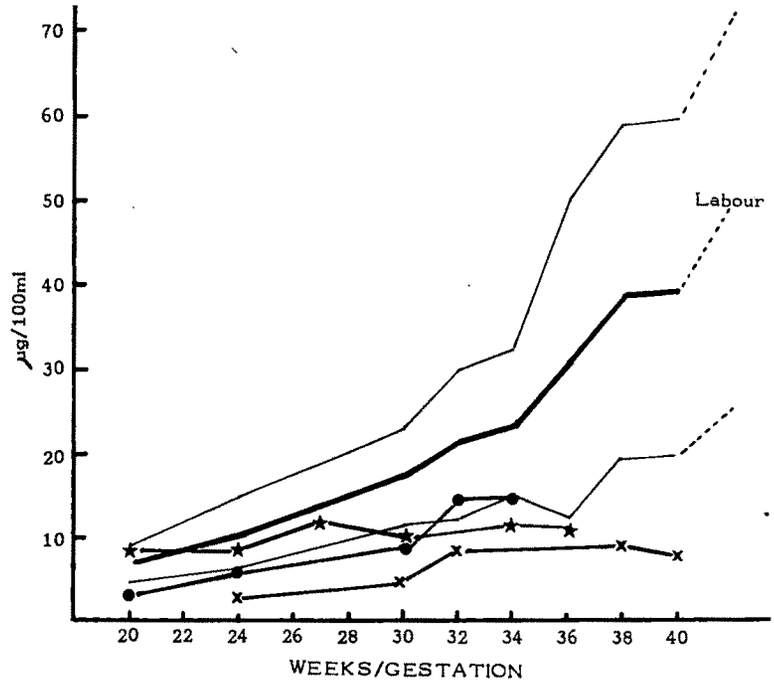


Fig. 4. Estriol levels in maternal plasma (normal ± one standard deviation) and in association with poor fetal growth and a case of premature labor at 30 weeks.

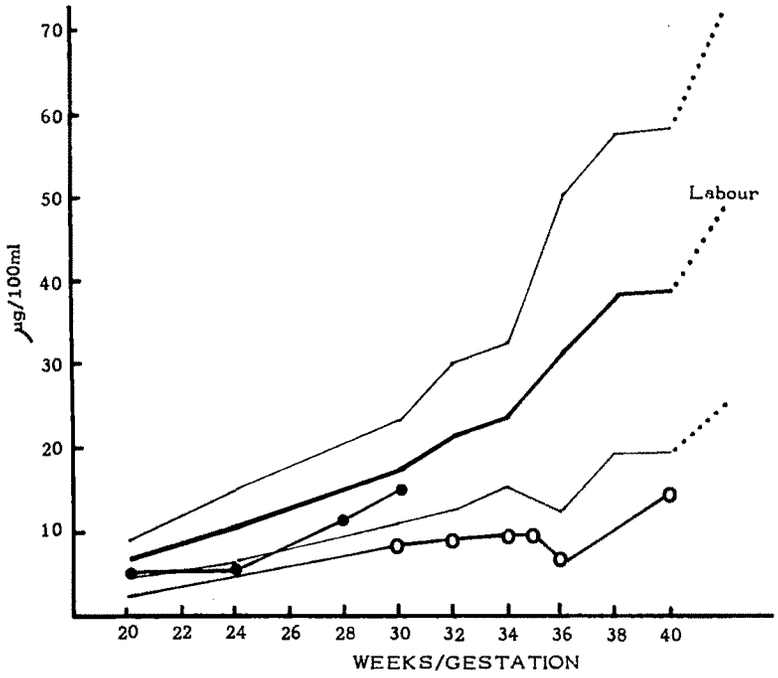


Fig. 5. Estriol levels in maternal plasma in pregnancy with anencephaly.

amniotic fluid are seen in congenital nephrosis[11] and in other central nervous system anomalies.

We have previously shown that there are two varieties of clinical anencephaly,[16] that occurring in first or second pregnancies (often associated with previous abortion), which tends to repeat several times, and that occurring sporadically in later pregnancies. Those who have had a damaged child before are at high risk and amniocentesis is justified, but the very large majority of women with an anencephalic or spina bifida–affected child have not had a previous child with the same condition. Therefore, screening by a blood test is essential. We have begun this screening and now take serum at all first clinic visits (10 to 20 weeks). High readings are expected in some 2 per cent of pregnancies, and in those amniocentesis is justifiable; however, anencephaly should be checked if possible by estriol excretion or by ultrasound and multiple pregnancy should be excluded.

In our own city, the rate of open spina bifida or anencephaly is likely to be about 7 per 1,000. Amniocentesis would be justifiable in cases with a high alpha fetoprotein level, since nearly one third of these are likely to have a child sufficiently abnormal to justify termination.

Preparation for screening test. Purified alpha fetoprotein was prepared by Mr. John Young in our laboratories from human fetal serum at 14 to 18 weeks' gestation.

Anti–alpha fetoprotein prepared in rabbits was obtained from Behringwerke. The radioimmunoassay was performed with the double-antibody method; the second antibody (a donkey anti-rabbit globulin) was obtained from Wellcome Laboratories.

Fig. 1 is a diagrammatic figure of the likely findings: (1) amniotic fluid, peak level at 14 weeks and by term may be barely detectable; (2) fetal serum, peak level also about 14 weeks of pregnancy and the fall is similar in time and degree to that in amniotic fluid; (3) maternal serum, levels are low at 14 weeks and rise to peak at 30 weeks, then decline.

Our own results in normal pregnancies (Fig. 2) show the expected pattern. It is important to see that in anencephaly (closed circles) very high readings before 20 weeks are clearly distinguishable from the normal. Levels change little in the presence of anencephaly so that by 30 weeks serum levels are very close to the upper range of normal.

Open circles show a twin pregnancy which at 30 weeks or so is indistinguishable from anencephaly. In diabetic pregnancy, readings are very low. Readings at 16 weeks should screen out anencephaly/spina bifida.

Progesterone. Progesterone produced by the placenta is essential to the fetus as a building block for the adrenal corticoids, and much profit might be gained from a study of fetal response to stress in association with the available levels of placental progesterone. High levels of corticosteroid of fetal origin are

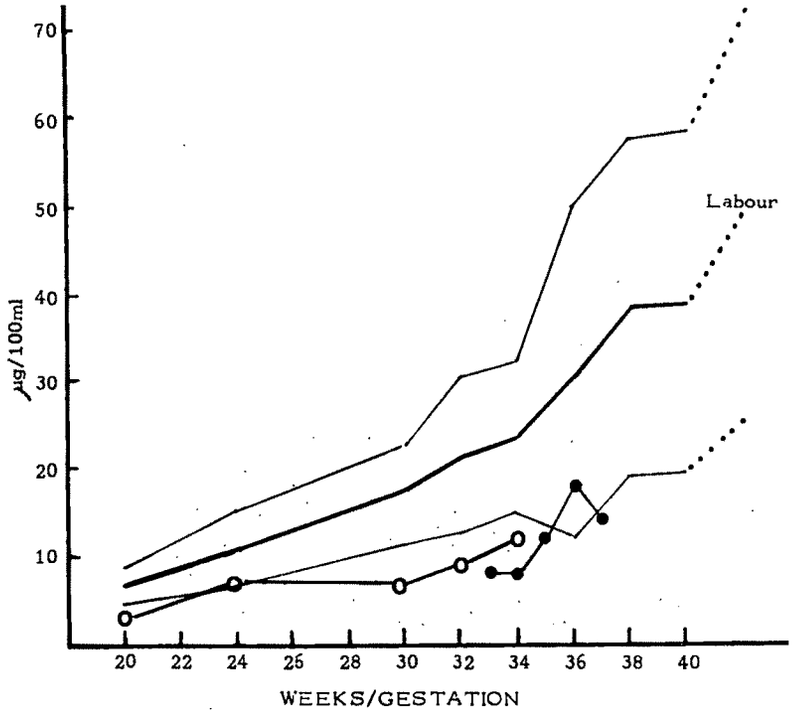


Fig. 6. Estriol levels in maternal plasma in a patient with diabetes.

associated with labor at term, and there must be an association with progesterone. Therefore, we are concerned with progesterone but still doubtful of its real role.

Our laboratory[9] demonstrated plasma levels running from 70 ng. at 20 weeks to a mean at 38 weeks of some 140 ng. although the scatter was wide (50 to 250 ng.). There was no rise or fall in the mean after 36 weeks.

Others[14] have shown mean levels rising from 50 to 150 ng. per milliliter from 20 to 34 weeks and then a clear fall to a mean of 100 ng. in the next few weeks until labor, but this fall was not found by all workers.[12]

Our most recent results are seen in Fig. 3. The mean level rises from 37 ng. per milliliter at 20 weeks steadily and *without a fall* to 154 ng. at 40 weeks (114 to 194 ng. ± one standard deviation).

A *diabetic* subject had very high readings in the blood, confirming the findings of others,[3] and some of the patients with *anencephalic* fetuses have low levels. Patients with *premature labor* had mixed readings.

The mean level of the maternal peripheral blood in the first stage of labor is a little lower than the term reading, but this is not uniform in the individual case as I[15] have shown previously.

Determination of the full significance of progesterone levels and those of its associated metabolites and of the ratio of estrogens (estradiol-17β and estriol) requires further study.

Estriol. The value of estimation of maternal urinary estriol in the antenatal care of women with hypertension syndromes in pregnancy,[7] in poor growth situations, and as an occasional monitor for anencephaly, congenital hepatitis, and the adrenal deficiency syndrome is undoubted.[6]

Estriol itself has no assignable function. It arises primarily - from maternal acetate by steps well known to us, from progesterone, and from maternal placental estradiol or estrone.

It is noted in studies from our biochemical medicine laboratory (Fig. 4) that levels of plasma estriol rise rapidly in late pregnancy. A rise has been shown by us previously with the use of gas-liquid chromatography (GLC) instead of radioimmunoassay.[5]

The clinical biochemists for this study used the technique originally described by Wilson[17] which is a modification of the technique of Gurpide and associates.[10] The antiserum itself is nonspecific, but adequate partitioning should remove estradiol, estrone, and other interfering estrogens. The assay as shown by GLC should then be specific for estriol and measure total conjugated, nonconjugated, protein-bound, and non–protein-bound hormones. The results shown here are roughly double those quoted by Wilson, and we are not entirely happy that our results are, in fact, totally specific but they are of practical clinical value.

Plasma estriol in this study rises rapidly for a mean of 6.4 μg per 100 ml. at 20 weeks to nearly 40 μg by term. In early labor, the level is even higher. Note the special

levels seen in those mothers with premature labor, poor growth of the fetus,[13] anencephaly (Fig. 5), and diabetes (Fig. 6). This promises to be a very useful tool.

It is interesting to note that alpha fetoprotein is likely to bind estrogen strongly and that the period of fall of alpha fetoprotein levels is close to that at which estrogen increases in concentration, again over a period of maximum blood volume.

Comment

This is a very sketchy outline of a preliminary report of an extensive study.

When we have complete studies, we should be able to correlate fetal well-being with the whole battery of tests as well as labor onset and the pattern of labor with the ranges and ratios of various hormones. Fetal levels at delivery will be available as will fetal blood gas information. Testosterone in the infant at birth will be followed into three-month testosterone levels and related to the stress situation. The infants will be followed up by Dr. Cecil Drillien and her research team. Perhaps in a year or so we may weave a series of stories from our material.

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Discussion

DR. JOHN B. MONTGOMERY. Philadelphia, Pennsylvania. I was impressed by Professor Walker's statistics which showed that perinatal deaths in Scotland have declined from 68 to 25 in each 1,000 births over a period of 30 years. This amazing decline is attributed to improvement in the health of the community, some decrease in parity, and improved obstetric care. This is in striking contrast to the rate in my own city of Philadelphia where the perinatal mortality rate has increased from 42 to 61 deaths per thousand births during the past 20 years. This dismal figure is, of course, associated with our shifting population, the increasing percentage of non-whites, an illegitimacy rate of 26 per cent, and other factors relating to population and geography.

By identifying the clinical cause of each perinatal death, Professor Walker has revealed that 72 per cent of the perinatal deaths in Dundee and in Glasgow are due to fetal deformity, antenatal hemorrhage, prematurity, and factors causing intrauterine fetal death. These losses cannot be prevented by obstetric care or intensive monitoring of labor.

Incidentally, the high rate of lethal fetal deformity (8.2 per 1,000 births) is apparently peculiar to Dundee and to Scotland. It contrasts sharply with the rate of 3 per 1,000 reported in Philadelphia in 1973.

Professor Walker accepts the use of intensive monitoring during labor but believes that this will save the lives of very few babies in Scotland. I suspect that its usefulness will prove to be far greater in our country, especially in those areas where we continue to em-

phasize childbirth without pain, elective induction of labor with oxytocin, heavy analgesia during labor, and operative delivery.

The real importance of Professor Walker's paper relates to his carefully planned cooperative study of the value of a variety of tests now available to us in studying the growth and development of the fetus. This area of study obviously could produce vitally important information regarding uncontrollable perinatal deaths which are of great concern to Professor Walker and to all obstetricians. The plan of study that he has presented appears to be logical and practical. The careful monitoring of these parameters of fetal growth and development throughout pregnancy in a sizable series of patients is a major effort involving many people and a great deal of work. The preliminary data that Professor Walker has presented from three important areas verify and expand some current concepts and indicate that much more extensive information will be available following completion of the current physiologic monitoring program. Correlation of those data with the quality of the baby produced and with the subsequent growth and development of the child should produce much new, valuable, and possibly startling information.

DR. E. STEWART TAYLOR, Denver, Colorado. Professor Walker has given the elements of modern prenatal care which comprise the new clinical science of perinatology. The elements that are most useful, I think, and in fact are almost indispensible to the operation of a modern obstetric unit are as follows: (1) serum estriol determinations, (2) amniotic fluid/creatinine levels, (3) lecithin/sphingomyclin ratios, (4) ultrasonic evaluation of the fetus for growth and development and for detection of congenital abnormalities in indicated situations, (5) the availability of genetic amniocentesis for study and detection of karyotype or chemical abnormalities, (6) availability of electronic and chemical fetal monitoring during labor, and (7) an intensive care nursery. Approximately 11 per cent of prenatal patients of an average service will require biochemical, ultrasonic, or genetic study during the prenatal course. These tests have to be done in specialized laboratories. Approximately 20 per cent of patients need to have electronic or biochemical monitoring during labor. We have found that 4 per cent of newborn infants in the State of Colorado require the availability of, or transfer to, an intensive care nursery. The staff must be composed of specially trained people.

The simple listing of these seven added essentials to modern obstetric care is not enough. In order to provide these services, a hospital must have at least one full-time consultant present who can interpret the data that come from the specialized laboratories and must have a team in the labor and delivery area that can evaluate and apply the information gained from the fetal monitoring. Most important of all, I think, is a well-equipped and well-staffed intensive care nursery facility where early diagnosis and treatment of diseases of the newborn infant can be carried out by nurses and pediatricians who are also neonatologists.

I enjoyed Professor Walker's review of the experience in Scotland very much. According to his statistics, only 25 per cent of the problem of perinatal loss is solvable by the acts of obstetricians; 75 per cent of the problem is social or genetic and beyond the scope of our instrumentations, laboratories, techniques, and decisions.

DR. KAMRAN S. MOGHISSI, Detroit, Michigan. I think that perhaps Dr. Taylor raised a very important point. We know that socioeconomic problems are having a great impact on perinatal deaths. Is Professor Walker able to tell us whether he can break down the socioeconomic subgroups contributing to each one of his reported factors in perinatal death? And, if so, how will he go about correcting such factors?

DR. A. BRIAN LITTLE, Cleveland, Ohio. I have an innate admiration for the British in that they have regionalized obstetric care. There are also attempts being made to regionalize obstetric care in this country. Although we are far behind, Professor Walker's presentation will add stimulus.

I would like to emphasize some points that he made. The first is cost. It has been simple in Britain and in Canada as health care costs gradually reached the gross national product and some decisions had to be made. For example, one of our colleagues recently referred to the large number of blood estriol determinations for prenatal diagnosis as "the billion dollar boondoggle." Therefore, we have to approach all newer tests with some reasonable type of evaluation to determine what is of value and what is not.

Also, most of us are not aware of the costs of obstetric care once proper care has been instituted for the patients that have high-risk problems, because they are concentrated in certain parts of the country and in certain parts of the cities. We have recently done a cost survey on 10 maternal and infant care projects across the country. The initial visit for a patient to one of these units will cost anywhere from $90 to $250. In addition, there has been an additional $40 to $50 for laboratory determinations that are being made on these patients. This appears to be a very high cost. When we also realize that the Robert Wood Johnson Foundation is now putting some ten or eleven million dollars into five centers to try to determine if regionalization if efficient, economic, and reasonable, I think we then can realize the importance of the work that has been presented to us today. We must solve some of these problems or we are going to price ourselves out of business.

DR. EDWARD J. QUILLIGAN, Los Angeles, California. I would like to make a couple of obvious comments. One is that the 23 per cent figure that has been alluded to is a very important figure. It tells us that one is going to

need to have tremendous numbers of patients before these techniques can actually be evaluated. This evaluation does need to be done, I think, desperately. Many of the techniques, including fetal heart rate monitoring and scalp sampling, have been almost accepted without complete evaluation which leads me to a question for Dr. Walker.

Are his patients going to be randomly assigned to these diagnostic modalities so that at an "x" period of time I can tell how patients have fared who have been monitored by one technique or another as opposed to those who have not?

DR. JAMES WALKER (Closing). I was expecting more criticism of some of our biochemistry.

In response to Dr. Montgomery's comments concerning the problems of perinatal death, we are fortunate that we have regionalization so that we can, in fact, examine deaths from a compact area. We are in our area about half a million people all together, and it is easy to keep an eye on what is happening and assess the clinical cause in each case of perinatal death. Interestingly, lethal deformity has gone up in the last few years.

Another new phenomenon also has occurred. Dr. Herrmann (and Dr. Moghissi) was talking the other day about the urban/rural story. We find in our rural districts that we now have a much higher perinatal death rate than in our urban districts. This is because of an increase in deformity rates and high prematurity rates in the rural population. This is quite new. The rise was bad enough to worry the local people there about obstetric standards until we showed them the causes. And, of course, it makes one feel a little helpless. Whether or not this has something to do with some new problem of the rural poor, I do not quite know. This method of using clinical assessment and clinical evaluation of death does help to pinpoint at least the obstetric clinical situations in which the problems are arising.

Dr. Taylor, I can assure you we have special nursery care which is of very high quality because our neonatal pediatrician was trained in Denver, as you know. He is a Scotsman who stayed for a long period in Denver and then came back, and we are delighted to have him. That is where we get our quality.

In this screening group of selected women that we are studying, we do not use invasive techniques. In other words, we are not using creatinine or lecithin/sphingomyelin ratios as part of the screening because we did not think this would be acceptable in otherwise perfectly normal patients. We do, of course, use such tests where they are clinically required.

The socioeconomic problems, of course, are terribly real. They show up, and this is re-emphasized as Dr. Little mentioned.

What are the problems now? How do we get better babies? Only something like 3 per cent of our perinatal risk is the *labor risk.* Labor is, of course, very highly and carefully monitored. But it is a tiny numerical group, 3 per cent of the total risk situation. The very, very high risk is in the babies who do not grow, the babies who are born too soon, and the babies who are abnormal. I do not know whether one should continue to be too worried about these; is it nature? We may well be coming down to an irreducible low, but, nevertheless, we should keep trying.

Before we consider the idea of feeding fetuses, we must get some measure of the problem. As you now know, amino acid studies suggest it is not always the mother who is at fault. The mother's levels are often high, but the baby's are not. So there are all sorts of problems.

Cost is part of the reason that brought this up. A Scot is canny, as you know. Half of this research was done by our own departmental (Obstetrics/Gynaecology) laboratories, and half was done by the routine hospital clinical chemists. They were worried that a whole range of tests would be asked for—without our junior staff knowing what the investigations meant and using them randomly. We were putting up costs as several thousand patients have routine tests done without any knowledge of the value of each test. This is why we did this study. It costs us something of the order of $2 for each estriol test.

Pure alpha fetoprotein is a very expensive material to obtain, and we have many vials which we made ourselves. This is one of the strange things. We have a very good young laboratory graduate technician who didn't know it was difficult to extract pure alpha fetoprotein so he just did so!

In this study, as currently designed, Dr. Quilligan, every woman has all tests done as a method of assessing value. Once this stage has been reached and we begin to assess value, we must get some answers with a "two-tailed" test or any other kind of statistical technique; we will do our best.

Varices of Pregnancy

Impending motherhood is a time for great expectation, and time for increased risk of varices in the lower extremities, especially with a second or subsequent pregnancy. The discomfort and disfigurement of varices are an unwelcome and perhaps unnecessary intrusion upon these special moments. Yet, increased intra-abdominal pressure, expanded blood volume and decreased physical activity may come to sharp focus during pregnancy predisposing to varices of the lower extremities.

Naturally, varicose veins become more severe in subsequent pregnancies and may reach the critical stage. Temporary valve incompetence associated with chronic venous distention lays the groundwork for vascular impairment of a very permanent nature, perhaps varicosities of the lower extremities for life.

A Jobst Pregnancy support applies external counter-pressures that oppose excessive venous pressures during pregnancy. Fatigue and discomfort are relieved. Edema is alleviated.[1] A Jobst support worn during the critical months of gestation, may prevent permanent damage to distended veins.[2]

Thompson reports, "We have preferred the Jobst stocking in our clinic. An elastic leotard produced by this company is particularly useful for varicose veins during pregnancy. Most patients are pleasantly surprised by the comforting support this garment gives."[3]



Write for the Jobst Program of Treatment for Varicosities Attendant to Pregnancy. You will receive a collection of clinical reprints on the subject and a full color, twelve-page monograph and bibliography describing the physiological fluid dynamics of external elastic compression. You might also like a twenty-eight-page patient education booklet, edited by a prominent obstetrician, entitled: Answering Your Questions About Varicose Veins During Pregnancy.

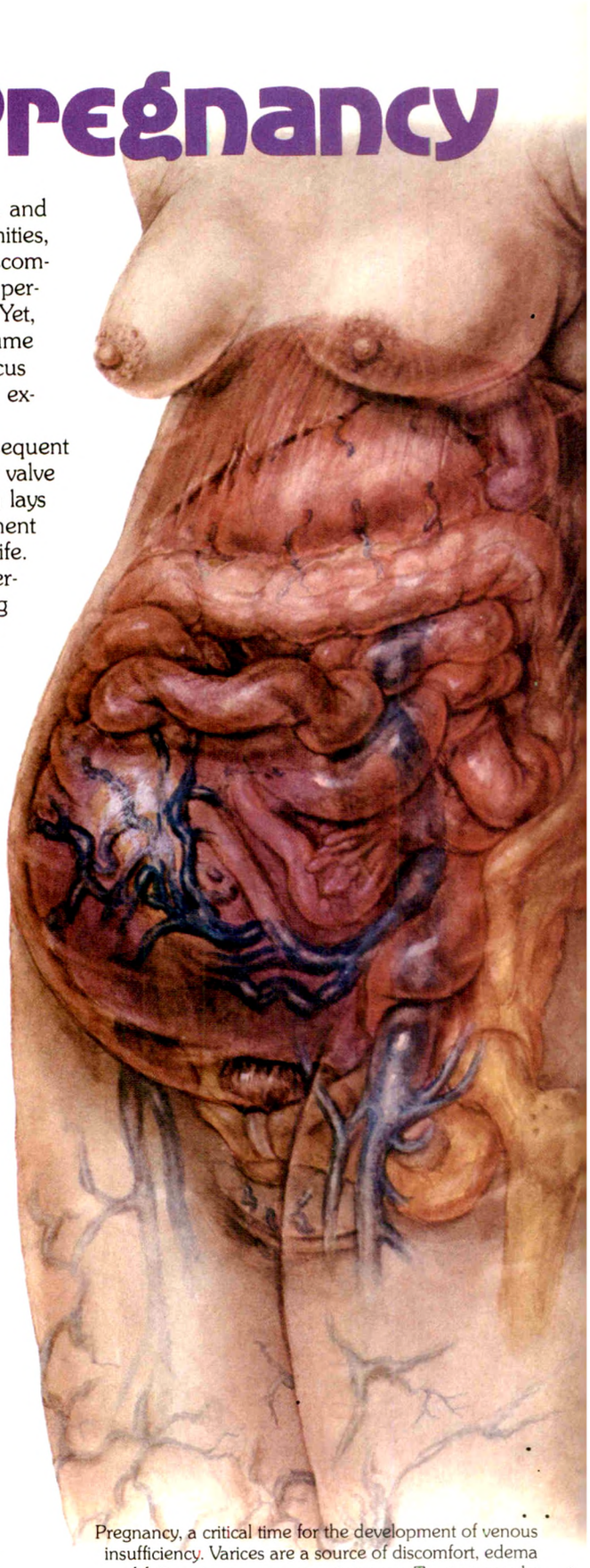
Varices of Pregnancy?
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1. Beninson J: Six years of pressure gradient therapy. A general survey. Angiology 12(1):38, 1961.

2. Fegan G: Varicose veins — compression sclerotherapy. Wm. Heinemann Medical Books Ltd., London 1967.

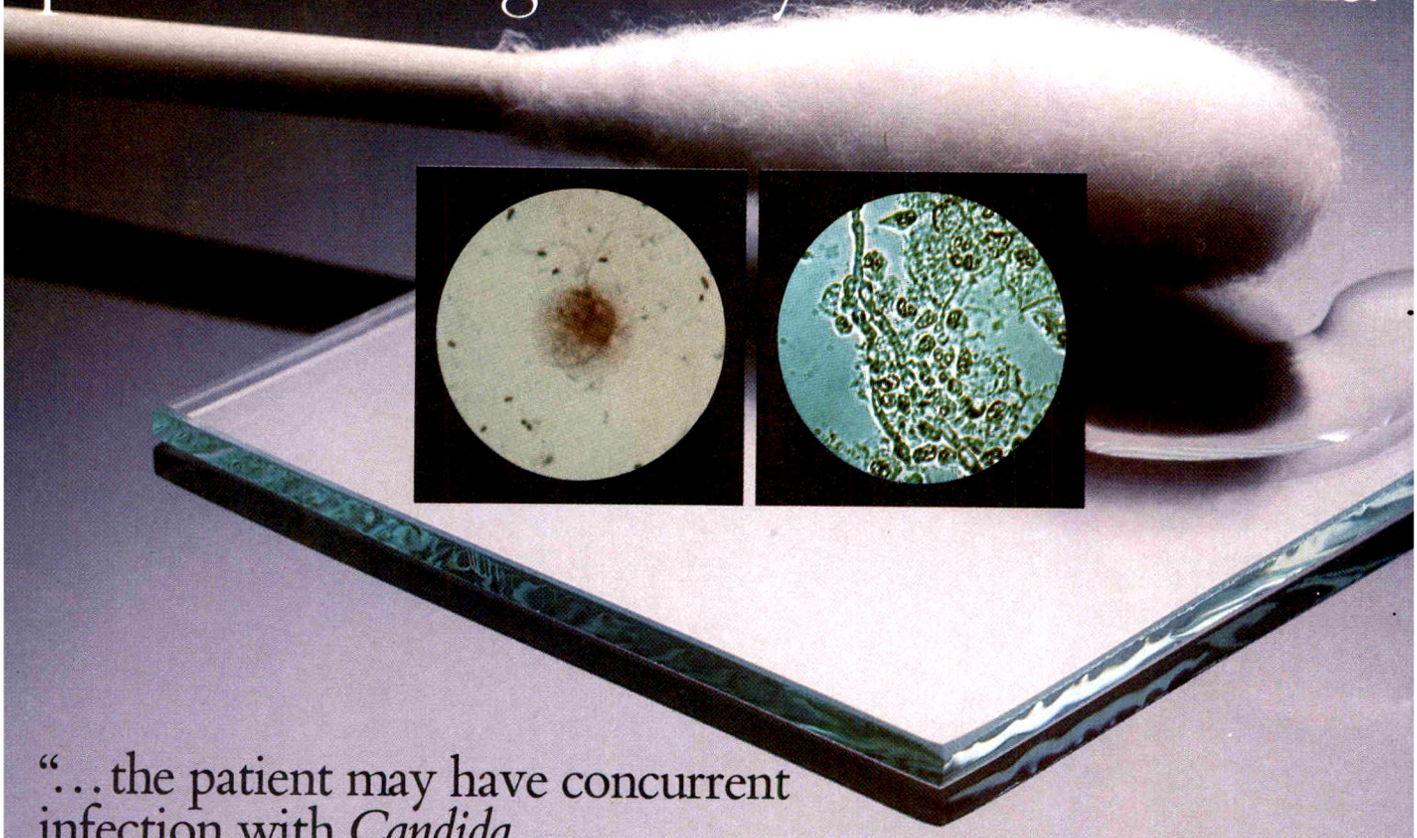
3. Thompson NW: The diagnosis and treatment of varicose veins. Univ of Mich Med Cntr Jnl 31 (1):11, 1965.



Pregnancy, a critical time for the development of venous insufficiency. Varices are a source of discomfort, edema and fatigue to many pregnant women. Temporary valve incompetence associated with chronic venous distention lays the groundwork for vascular impairment of a very permanent nature. Perhaps, disfigurement for life.

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When a mixed infection causes a vaginal problem...two agents may be better than one.



"...the patient may have concurrent infection with *Candida*... as well as *Trichomonas vaginalis*."[1]

Burning, erythema, itching and discharge are common symptoms of a variety of vaginal infections. It is therefore important to realize that vaginal infection can be mixed. The coexistence of *Candida* and *Trichomonas vaginalis*, for example, is not uncommon.

A topical fungicide will usually control the yeast infection of candidiasis.

Flagyl® (metronidazole) is the recognized treatment of choice for trichomonal vaginitis, which is often characterized by a bubbly, thin, yellow to light green or grey discharge. In addition, secondary symptoms of pruritus vulvae, soreness, irritation, burning, dyspareunia, offensive odor and, occasionally, urinary frequency may occur. The systemic action of Flagyl may usually be relied upon to eliminate trichomonads from the vagina, the paraurethral and Bartholin's glands, and the bladder as well.

Therapy of choice for women: One 250-mg. Flagyl tablet orally three times daily for ten days in conjunction with treatment of her male partner, when he has been shown to harbor the organism.

The only choice for infected men: One 250-mg. Flagyl tablet orally two times daily for ten days concurrent with treatment of his infected female partner. Topical medications are useless in men. Only the systemic action of Flagyl can eliminate trichomonads entrenched in the male genitourinary tract.

1. Cibley, L. J. and Kasdon, S. C.: A New Look at Vulvovaginitis, ed. 2, Chicago, Searle & Co., 1972, p. 11.

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Make sure trichomoniasis isn't left behind.

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(metronidazole)

Indications: For the treatment of trichomoniasis in both male and female patients and in the sexual partners of patients with a recurrence of the infection provided trichomonads have been confirmed by appropriate laboratory procedures (wet smear or culture). The oral tablets are indicated also for acute intestinal amebiasis (amebic dysentery) and amebic liver abscess.

Contraindications: Evidence or history of blood dyscrasia, active organic disease of the CNS, the first trimester of pregnancy and a history of hypersensitivity to metronidazole.

Warnings: Flagyl should not be used in the first trimester of pregnancy (see *Contraindications).* Use with discretion during the second and third trimesters of pregnancy and restrict to those pregnant patients not cured by topical measures. Flagyl (metronidazole) is secreted in the breast milk of nursing mothers. It is not known whether this can be injurious to the newborn.

Note: Tumorigenicity Studies in Rodents. Metronidazole has shown evidence of tumorigenic activity in a number of studies involving chronic, oral administration in mice and rats.

Most prominent among the effects in the mouse was the promotion of pulmonary tumorigenesis. This has been observed in all five reported studies in that species, including one study in which the animals were dosed on an intermittent schedule (administration during every fourth week only). The published results of one of the mouse studies indicate increases in the incidence of malignant lymphomas as well as pulmonary neoplasms associated with lifetime feeding of the drug. All these effects are statistically significant.

Two long-term toxicity studies in the rat have been completed. There was a statistically significant increase in the incidence of various neoplasms, particularly mammary tumors, among female rats administered metronidazole over that noted in the concurrent female control groups.

Unpublished results of a lifetime tumorigenicity study in hamsters have been reported to be negative.

Precautions: Mild leukopenia has been reported during Flagyl use; total and differential leukocyte counts are recommended before and after treatment with the drug, especially if a second course is necessary. Avoid alcoholic beverages during Flagyl therapy because abdominal cramps, vomiting and flushing may occur. Discontinue Flagyl promptly if abnormal neurologic signs occur. Exacerbation of moniliasis may occur. In amebic liver abscess, aspirate pus during metronidazole therapy.

Adverse Reactions: Nausea, headache, anorexia, vomiting, diarrhea, epigastric distress, abdominal cramping, constipation, a metallic, sharp and unpleasant taste, furry tongue, glossitis and stomatitis possibly associated with a sudden overgrowth of *Monilia,* exacerbation of vaginal moniliasis, an occasional reversible moderate leukopenia, dizziness, vertigo, incoordination and ataxia, numbness or paresthesia of an extremity, fleeting joint pains, confusion, irritability, depression, insomnia, mild erythematous eruptions, "weakness," urticaria, flushing, dryness of the mouth, vagina or vulva, pruritus, dysuria, cystitis, a sense of pelvic pressure, dyspareunia, fever, polyuria, incontinence, decrease of libido, nasal congestion, proctitis, pyuria and darkened urine have occurred in patients receiving the drug. Patients receiving Flagyl may experience abdominal distress, nausea, vomiting or headache if alcoholic beverages are consumed. The taste of alcoholic beverages may also be modified. Flattening of the T wave may be seen in ECG tracings.

Dosage and Administration: For Trichomoniasis. *In the female:* One 250-mg. tablet orally three times daily for ten days. Courses may be repeated if required in especially stubborn cases; in such patients an interval of four to six weeks between courses and total and differential leukocyte counts before, during, and after treatment are recommended. Vaginal inserts of 500 mg. are available for use, particularly in stubborn cases. *When the vaginal inserts are used,* one 500-mg. insert is placed high in the vaginal vault each day for ten days and the oral dosage is reduced to two 250-mg. tablets daily during the ten-day course of treatment. Do not use the vaginal inserts as the sole form of therapy. *In the male:* Prescribe Flagyl only when trichomonads are demonstrated in the urogenital tract, one 250-mg. tablet two times daily for ten days. Flagyl should be taken by both partners over the same ten-day period when it is prescribed for the male in conjunction with the treatment of his female partner.

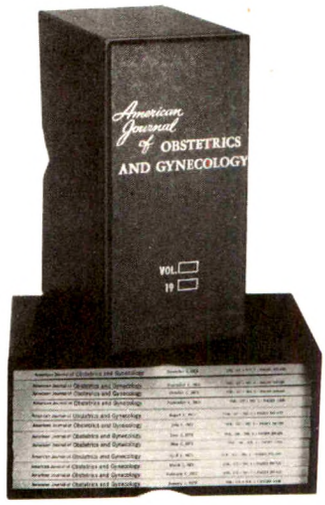
For Amebiasis. *Adults:* For acute intestinal amebiasis, 750 mg. orally three times daily for 5 to 10 days. For amebic liver abscess, 500 to 750 mg. orally three times daily for 5 to 10 days. *Children:* 35 to 50 mg./kg. of body weight/24 hours, divided into three doses, orally for ten days.

Dosage Forms: Oral tablets, 250 mg. Vaginal inserts, 500 mg.; inactive ingredients are lactose, potato starch, polyvinyl alcohol and hydrogenated castor oil.

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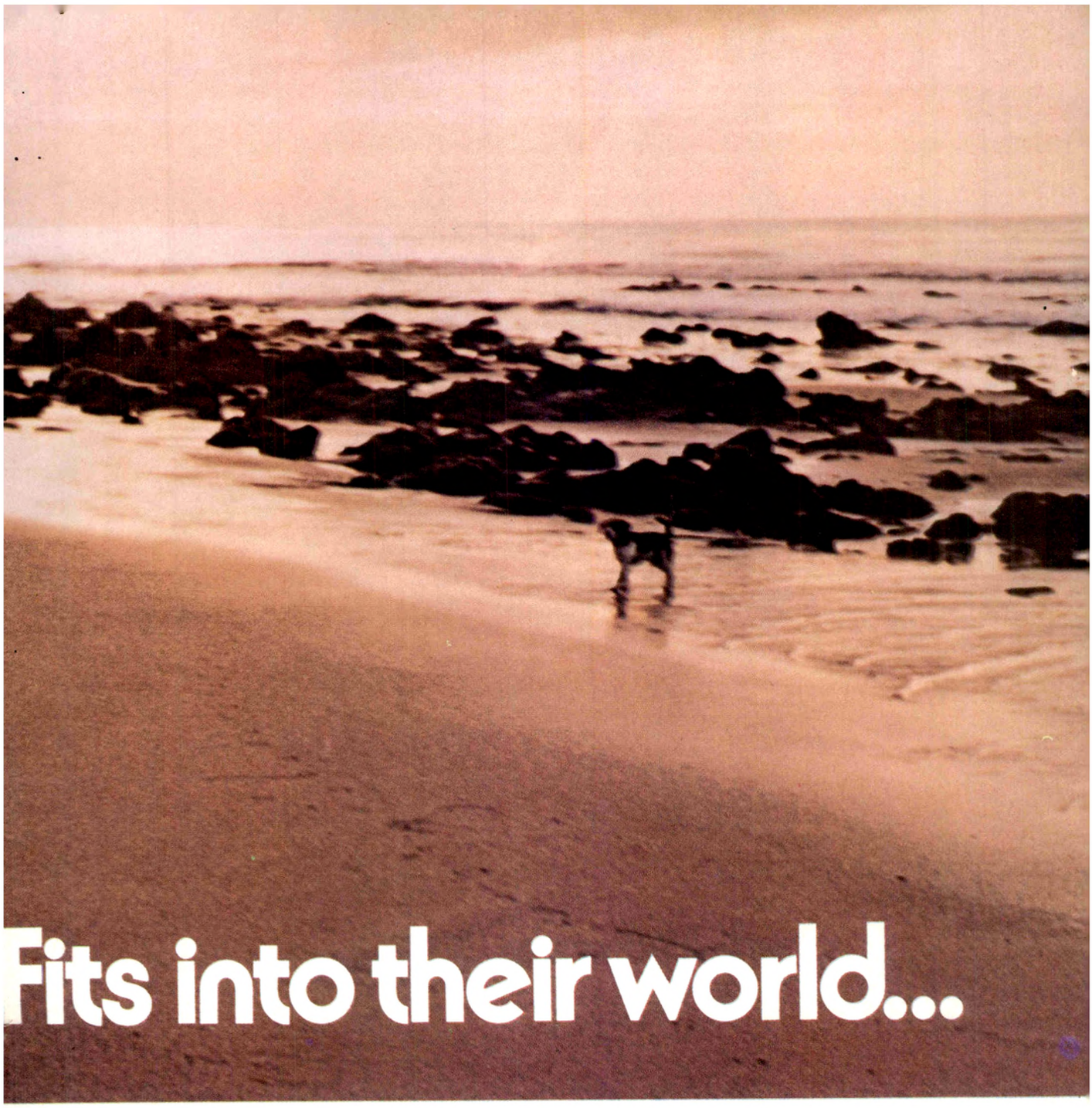
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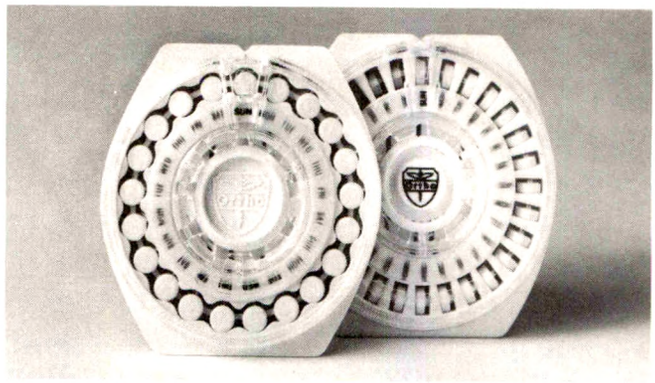
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†Serious as well as minor conditions have been reported following the use of oral contraceptives. These conditio include thromboembolic disease. The physician should alert to the earliest manifestations of any symptoms of serious disease and discontinue oral contraceptive there when appropriate. The physician should be fully aware of the complete Prescribing Information for this produc

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Actions: ORTHO-NOVUM 1/50□21 and ORTHO-NOVUM 1/50□28 act through the mechanism of gonadotrophin suppression, i.e., through the estrogenic and progestational actions of the ingredients. Alterations in cervical mucus and in the endometrium may also contribute to the efficacy of ORTHO-NOVUM 1/50□21 and ORTHO-NOVUM 1/50□28.

Special note: Oral contraceptives have been marketed in the United States since 1960. Reported pregnancy rates vary from product to product. The effectiveness of the sequential products appears to be somewhat lower than that of the combination products. Both types provide almost completely effective contraception.

An increased risk of thromboembolic disease associated with the use of hormonal contraceptives has now been shown in studies conducted in both Great Britain and the United States. Other risks, such as those of elevated blood pressure and reduced tolerance to carbohydrates, have been reported and appropriate tests should be conducted to monitor these during oral contraceptive therapy. Liver disease has also been reported, and the physician should be alert to its earliest manifestations.

Long-term administration of both natural and synthetic estrogens in subprimate animal species in multiples of the human dose increases the frequency for some animal carcinomas. These data cannot be transposed directly to man. The possible carcinogenicity due to the estrogens can neither be affirmed nor refuted at this time. Close clinical surveillance of all women taking oral contraceptives must be continued.

Indication: Contraception.

Contraindications: 1. Thrombophlebitis, thromboembolic disorders, cerebral vascular disease, or a past history of these conditions. 2. Markedly impaired liver function. 3. Known or suspected carcinoma of the breast. 4. Known or suspected estrogen-dependent neoplasia. 5. Undiagnosed abnormal genital bleeding. 6. Known or suspected pregnancy.

Warnings: 1. The physician should be alert to the earliest manifestations of thrombotic and thromboembolic disorders. thrombophlebitis, cerebrovascular disorders including hemorrhage, pulmonary embolism and retinal thrombosis. Should any of these occur or be suspected, the drug should be discontinued immediately.

Retrospective studies of morbidity and mortality in Great Britain and studies of morbidity in the United States have shown a statistically significant association between thrombophlebitis, pulmonary embolism and cerebral vascular disease, occlusive or hemorrhagic, and the use of oral contraceptives. There have been three principal studies in Great Britain[1-3] leading to these conclusions and three in this country.[4-7] The estimate of the relative risk of thromboembolism in the study by Vessey and Doll[3] was about sevenfold, while the United States studies found relative risks of 4.4 to 11, meaning that the users are several times as likely to undergo thromboembolic disease without evident cause as non-users.

In May, 1974, the Royal College of General Practitioners in England[8] issued an interim report of its continuing large-scale prospective study comparing a user group to a non-user group. This study in its interim analysis states, "A statistically significant higher rate of reporting of cerebrovascular accidents in Takers is evident, but the numbers are too small to justify an estimation of the degree of risk." The study also reported a higher incidence of superficial and deep vein thrombosis in users as compared to non-users. The risk of superficial and deep vein thrombosis was reported to be lower in women using 50 mcg estrogen preparations.

The Sartwell study[4] indicated that the risk did not persist after discontinuation of administration. Both the Sartwell and the Royal College studies indicated that the degree of risk was not associated with duration of treatment.

In a collaborative American study[5,6] of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the relative risk of thrombotic stroke was 4.1 to 9.5 times greater in users than in non-users. A comparable estimate for hemorrhagic stroke was 2.0.

None of the American studies was designed to evaluate a difference between products. However, the Sartwell study[4] suggested that there might be an increased risk of thromboembolic disease in users of sequential products.

Other retrospective studies[9,10] have reported an increased risk of post-surgery thromboembolic complications in oral contraceptive users. It has been recommended that therapy be discontinued at least one month prior to elective surgery.

2. Discontinue oral contraceptive medication if there is: gradual or sudden partial or complete loss of vision; proptosis or diplopia; onset or aggravation of migraine or development of headache of a new pattern which is recurrent, persistent or severe; papilledema; or any evidence of retinal vascular lesions.

3. Fetal abnormalities have been reported to occur in the offspring of women who have taken progestogens and/or estrogens during pregnancy.[11,12] The safety of ORTHO-NOVUM 1/50□21 or ORTHO-NOVUM 1/50□28 in pregnancy has not been demonstrated. Pregnancy should be ruled out before initiating or continuing the contraceptive regimen. Pregnancy should always be considered if withdrawal bleeding does not occur.

4. A small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The long-range effect to the nursing infant cannot be determined at this time.

5. Hepatic lesions (adenomas, hepatomas, hamartomas, regenerating nodules, etc.), occasionally fatal, have been reported in women on oral contraceptives. Such lesions may present as an abdominal mass or with the signs and symptoms of an acute abdomen. These lesions should be considered if the patient has abdominal pain or evidence of intra-abdominal bleeding. This has been reported in short-term as well as long-term users of oral contraceptives.

Precautions: 1. A thorough history and physical examination should be performed before prescribing oral contraceptives and periodically during their administration and should include special reference to breasts and pelvic organs, including Papanicolaou smear since estrogens have been known to produce tumors, some of them malignant, in five species of subprimate animals. 2. Endocrine and possibly liver function tests may be affected by treatment with ORTHO-NOVUM 1/50□21 or ORTHO-NOVUM 1/50□28. Therefore, if such tests are abnormal in a patient taking ORTHO-NOVUM 1/50□21 or ORTHO-NOVUM 1/50□28, it is recommended that they be repeated after the drug has been withdrawn for two months. 3. Under the influence of estrogen-progestogen preparations, pre-existing uterine fibromyomata may increase in size. 4. Because these agents may cause some degree of fluid retention, conditions which might be influenced by this factor, such as epilepsy, migraine, asthma, cardiac or renal dysfunction, require careful observation. 5. In breakthrough bleeding, and in all cases of irregular bleeding per vaginam, nonfunctional causes should be borne in mind. In undiagnosed bleeding per vaginam, adequate diagnostic measures are indicated. 6. Patients with a history of psychic depression should be carefully observed and the drug discontinued if the depression recurs to a serious degree. 7. Any possible influence of prolonged ORTHO-NOVUM 1/50□21 or ORTHO-NOVUM 1/50□28 therapy on pituitary, ovarian, adrenal, hepatic or uterine function awaits further study. 8. A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. The mechanism of this decrease is obscure. For this reason, diabetic patients should be carefully observed while receiving ORTHO-NOVUM 1/50□21 or ORTHO-NOVUM 1/50□28 therapy. 9. The age of the patient constitutes no absolute limiting factor, although treatment with ORTHO-NOVUM 1/50□21 or ORTHO-NOVUM 1/50□28 may mask the onset of the climacteric. 10. The pathologist should be advised of ORTHO-NOVUM 1/50□21 or ORTHO-NOVUM 1/50□28 therapy when relevant specimens are submitted. 11. Susceptible women may experience an increase in blood pressure following administration of contraceptive steroids. 12. Cholestatic jaundice has been reported in users of oral contraceptives. If this occurs, ORTHO-NOVUM 1/50□21 or ORTHO-NOVUM 1/50□28 should be discontinued. This condition is more likely to occur in patients who have experienced cholestatic jaundice of pregnancy. Patients with a history of cholestatic jaundice of pregnancy should be carefully observed during ORTHO-NOVUM 1/50□21 or ORTHO-NOVUM 1/50□28 therapy.

Adverse reactions observed in patients receiving oral contraceptives: A statistically significant association has been demonstrated between use of oral contraceptives and the following serious adverse reactions: thrombophlebitis, pulmonary embolism, cerebral thrombosis and hemorrhage, gallbladder disease.

Although available evidence is suggestive of an association, such a relationship has been neither confirmed nor refuted for the following serious adverse reactions: neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis, hepatic lesions with or without intra-abdominal bleeding.

The following adverse reactions are known to occur in patients receiving oral contraceptives: nausea, vomiting, gastrointestinal symptoms (such as abdominal cramps and bloating), breakthrough bleeding, spotting, change in menstrual flow, amenorrhea during and after treatment, edema, chloasma or melasma, which may persist, cholestatic jaundice, migraine, rash (allergic), mental depression, change in weight (increase or decrease), breast changes (tenderness, enlargement and secretion), changes in cervical erosion and cervical secretions, suppression of lactation when given immediately post-partum, rise in blood pressure in susceptible individuals.

Although the following adverse reactions have been reported in users of oral contraceptives, an association has been neither confirmed nor refuted: anovulation post-treatment, which tends to occur more frequently in women with a history of menstrual disorders, premenstrual-like syndrome, changes in libido, changes in appetite, cystitis-like syndrome, headache, intolerance to contact lenses, nervousness, dizziness, fatigue, backache, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruptions, itching, vaginitis.

The following laboratory results may be altered by the use of oral contraceptives: hepatic function—increased sulfobromophthalein retention and other tests; coagulation tests—increase in prothrombin, Factors VII, VIII, IX and X; decrease in anti-thrombin III; increase in platelet aggregability; thyroid function—increase in PBI, and butanol-extractable protein-bound iodine and decrease in T^3 uptake values; metyrapone test; pregnanediol determination.

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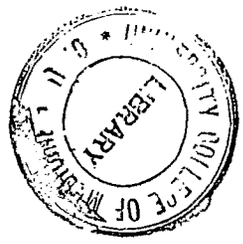
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FETUS, PLACENTA, AND NEWBORN

Management of isoimmune neonatal thrombocytopenia

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Two infants, who presented at birth with isoimmune thrombocytopenic purpura, are the basis for this report. The problems confronting the physician in treating an affected infant, as well as in the management of subsequent pregnancies after an infant with isoimmunization has been delivered, are discussed. In view of the small but serious risk of intracranial hemorrhage during the birth process in these infants, delivery by cesarean section is advocated for all pregnancies known to be at risk i.e., after a previous infant has been shown to be affected.

WHEN THROMBOCYTOPENIA is noted at birth in an infant whose mother has a normal platelet count and no known platelet defect, an isoimmune etiology may be hypothesized. Although there have been many reports of this phenomenon,[1-3] the incidence is relatively low, approximately 1 or 2 per 10,000 births.[3-4] Pearson and associates[1] estimated that 20 per cent of all cases of neonatal thrombocytopenia fall into this category. In these infants the disease process is suspected of being analogous to that seen with red cell antigens in erythroblastosis fetalis. First-born infants are likely to be affected; in fact, Shulman and associates[3] reported that in a group of 51 suspected cases of neonatal isoimmune thrombocytopenic purpura, 49 per cent were in the first-born child.

Although the incidence of serious hemorrhage in infants with thrombocytopenia is low,[5] the greatest risk of intracranial bleeding would appear to be during the birth process and perinatal management may be critical. Two siblings will be presented who were found to have purpura at birth. Both infants have neurologic sequelae. The probability that this damage was secondary to intracranial hemorrhage during the birth process has prompted a re-evaluation of pre- and postnatal management of infants in whom isoimmune thrombocytopenia is a possibility.

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Case reports

Case 1. A male infant, birth weight 3,610 grams, was delivered on May 18, 1971, to a gravida 1, 25-year-old mother, who had a negative serology and whose blood type was B-Rh positive. She had been in good health and had never received blood transfusions. Primary dysfunctional labor was augmented by pitocin infusion and led to a vertex delivery. The infant had Apgar scores of 8, 9, and 10 at 1, 2, and 5 minutes. A purpuric.

rash present at birth over the shoulders and in the inguinal area was noted to increase in the first few moments of life. The infant appeared vigorous and had no hepatosplenomegaly. Laboratory evaluation revealed the following: blood type, B-Rh positive; direct Coombs test, negative; hemoglobin, 17 Gm.; platelets, 22,000; WBC, 25,000; polymorphonuclear leukocytes (poly.), 66 (0-2-64); lymphocytes (lymph.), 30; monocytes (mono.), 2; eosinophils (eos.), 2; reticulocyte (retic.) count, 11.3 per cent (Table I). Screening tests for coagulation abnormalities were normal for age of patient. Bone marrow aspiration showed a normal number of megakaryocytes. Urinalysis was normal. Neonatal infection was suspected but could not be bacteriologically proven. Blood culture, serology, toxoplasmosis titer, and rubella titer were all negative. Skeletal survey and skull x-rays were normal. Urinary amino acid screening was normal and serum IgM titer was normal (12 mg. per 100 ml.).

At 60 hours of age slight jaundice was noted and a peak total bilirubin level of 11.4 mg. per 100 ml. was observed on the fourth day. A repeat hemogram on the third day showed the following: hemoglobin, 16.9 Gm.; WBC, 10,500; retic., 7.7 per cent; platelets, 26,000. No medication was given. By the fifth day the petechiae had cleared; when the baby was discharged on the sixth day, his physical and neurologic examinations were normal. The hemogram at discharge was as follows: hemoglobin, 15.8 Gm.; WBC, 10,300; poly., 34 (0-2-32); lymph., 57; mono., 6; myelo., 3; platelets, 90,000.

Blood was drawn from the mother and the infant on the third day following delivery for performance of platelet Factor III antibody test.[6] An elevated antibody titer of 1:24 was found in the mother's blood and 1:80 in that of the infant (normal $< 1:4$). Simultaneous platelet counts in the mother and infant were 260,000 and 20,000, respectively. At age 4 months, the infant had a hemoglobin of 11.3 Gm. and a platelet count of 220,000.

At 8 months of age he was readmitted to the hospital due to infantile spasms and a possible mild right hemiparesis. The platelet count was normal at this time. The child has been followed by a pediatric neurologist and found to have psychomotor retardation and poor language development at age 3 years.

Case 2. A second male child, birth weight 3,540 grams, was delivered to the same mother on October 17, 1972, at term, after an uneventful pregnancy. No platelet antibodies had been detected in the maternal blood when tested 4 weeks prior to delivery. This fact led to the decision to allow vaginal delivery rather than to perform a cesarian section. Labor lasted 8 hours and the membranes ruptured 3 hours before delivery. The infant was biochemically monitored during labor with serial scalp blood samples. A sustained bradycardia was noted, and for this reason delivery was assisted by the application of midforceps. The infant had Apgar scores of 8, 8, and 9 at 1, 2, and 5 minutes. There was acrocyanosis and petechiae were noted over the trunk and groin. Molding and a large caput succedaneum were also noted. The anterior fontanelle measured 2 by 3 cm. and was flat. No heart murmur was heard. The liver and spleen were not enlarged. The petechiae were noted to increase and the infant was transferred to the newborn intensive-care unit. Laboratory evaluation showed the following: blood type, AB-Rh positive; direct Coombs test, negative; hemoglobin, 15.4 Gm.; hematocrit, 43.5 per cent; WBC, 14,100; RBC, 4.09 million; poly., 49 (1-7-41); eos., 4; lymph., 37; mono., 9; myelo., 1; platelets, 15,000 (Table I).

Despite the fact that platelet antibodies had not been demonstrated in the maternal blood 4 weeks prior to this infant's delivery, a presumptive diagnosis of platelet isoimmunization was made on the basis of findings in the previous pregnancy. This diagnosis was later supported by the finding of platelet Factor III antibody in a titer of 1:20 in the infant's blood at birth. Oral prednisone was started in a dose of 10 mg. per day.

Since the first-born infant had exhibited evidence of CNS injury, probably secondary to perinatal intracranial bleeding, it was deemed advisable to do an exchange transfusion on the second infant in an attempt to correct the thrombocytopenia. This was performed at 5 hours of age. Since platelet-matched blood was not readily obtainable, it was elected to perform the exchange transfusion, using 500 c.c. of blood obtained from the mother. The procedure was carried out without difficulty and without ill effects to either the mother or the infant. In this case no problems were presented by the red cell antigens, the mother being B-Rh positive and the baby AB-Rh positive.

Platelet counts and other peripheral blood findings in the infant during the first 10 days of life are shown in Table I.

At 24 hours of life there were no new petechiae and no bleeding. There was, however, intermittent bradycardia. Neurologic evaluation was inconclusive in the light of equivocal head control and questionable hypotonia, but the neurologic consultants felt that no further studies were indicated. The child improved over the next 4 days and was discharged to his home at 7 days of age. Prednisone was gradually tapered and discontinued 16 days after discharge. The platelet count remained normal.

The infant has been followed as an out-patient by a pediatric neurologist and at two years of age has significantly delayed motor milestones and no speech development.

Comment

Two main problems confront the physician who is faced with a patient in whom isoimmune neonatal purpura is suspected: one concerns the treatment of

Table I. Hematologic findings in the neonatal period

Date	Hgb.	WBC	Differential					Platelets	Retic. (%)
			Poly.	Lymph.	Eos.	Mono.	Myelo.		
Case no. 1—1971:									
5/18	17.0	25,000	66 (0-2-64)	30	2	2		22,000	11.2
5/19	16.4							20,000	
5/20	16.4							22,000	
5/21	16.9	10,500	44	45	7	4		26,000	7.7
5/22	16.9							70,000	
5/23									
5/24	15.8	10,300	34 (0-2-32)	57		6	3	90,000	
Case no. 2—1972:									
10/17	15.4	14,100	49 (1-7-41)	37	4	9	1	15,000	*
10/18	14.4	14,600						116,600	
10/19	15.5							128,000	
10/20								118,000	
10/21								52,000	
10/22								52,000	
10/23								184,000	
10/24								215,000	
10/27								256,000	

*Eight nucleated RBC per 100 WBC.

the affected infant and the other concerns the management of subsequent pregnancies in a mother who has delivered one affected infant.

The course of isoimmune thrombocytopenia in the infant is variable, ranging from benign resolution in 2 to 3 weeks to a 12 to 14 per cent mortality rate, secondary to intracranial hemorrhage.[1–5] In addition, there can be neurologic sequelae in those infants in whom clinically inapparent or seemingly minor bleeding may have occurred. This would seem to have been the case in the two infants reported here. Various modalities of treatment have been used. Steroids have been given prenatally to the mother or at birth to the infant.[1–3]

Exchange transfusion with RBC-compatible blood does aid in the removal of circulating antibody, but, unless the blood is also platelet-type specific, it will not appreciably increase the level of circulating platelets. Platelet typing is not yet readily available and for this reason the use of maternal platelets which would not be affected by the platelet antibody has been advocated.[2, 4] In the second infant described, the platelet count rose immediately after the exchange transfusion to 116,600 per cubic millimeter. This rise was due to platelets received from the mother and persisted until the fourth day. The dip in platelet counts on the fourth and fifth days probably reflected disappearance of maternal platelets. Exchange transfusion with maternal whole blood is feasible where mother and infant have compatible RBC antigens and avoids the technical problem of separating off the platelets.

Various types of platelet antibodies have been detected in isoimmune neonatal thrombocytopenia. Some of these are unique to platelets and others are shared by platelets and leukocytes. PL^{A1} antigen, which is unique to platelets, has been implicated most frequently.[1–4] Since 97 to 98 per cent of people are positive for PL^{A1}, the chances of obtaining a compatible PL^{A1}-negative donor on a routine basis are slight. In addition, platelet typing is cumbersome and not readily available at present. Those antigens which platelets share with leukocytes, the HLA types, can be identified more easily, but they are not frequently implicated and appear to be found in the milder cases of immunization.[7]

With the methods of detecting platelet antibodies that are currently available, one can usually establish retrospectively that platelet isoimmunization may have occurred in an affected infant. Since, however, the greatest risk of intracranial hemorrhage occurs during the birth process, a major problem remains of prediction of the outcome of a given pregnancy. This difficulty is increased by virtue of the fact that platelet isoimmunization occurs most often in first pregnancies. Once a woman has delivered an affected infant, the physician can be alerted to the possibility of subsequent children being at risk. Serial antibody titers in the mother during pregnancy may be helpful, but it has

been shown that the level of maternal antibodies does not correlate with the severity of the disease in the infant.[3, 8] Furthermore, when antibodies are found in the mother at the time of delivery of an affected child, they can usually be detected for only about 2 weeks postpartum and, as in our second patient, may not be found to be elevated antepartum even when the infant is affected. The analogy to Rh sensitization may be used in interpreting these antibody titer findings. It has been found[7] in Rh sensitization that if a titer has been rising during pregnancy and then dips 4 to 6 weeks before delivery, the infant is likely to be affected and its red cells in effect have "soaked up" the antibodies. Similarly, if titers were elevated from a previous pregnancy and remained so during a subsequent pregnancy, the infant is likely to be "D" negative and unaffected.

It is possible that the lack of platelet antibodies in maternal blood 4 weeks prior to the delivery of the second child reported here may have been an indication of the fact that the infant was affected. Since this was the only titer obtained during this pregnancy, we do not know if there might have been an earlier elevation.

While the incidence of severely affected infants is low, when neurologic damage occurs, it is devastating. In the absence of reliable indicators for identifying platelet isoimmunization prior to delivery not much can be done to protect a first affected infant. When a previous pregnancy has resulted in a baby who had isoimmune thrombocytopenic purpura, however, it would seem prudent to consider all subsequent pregnancies at risk. In such a situation, serial testing for platelet antibody in maternal serum may be helpful, but delivery by cesarian section would appear to be the only way of protecting the potentially affected infant from the trauma of vaginal delivery.

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Cortisol in amniotic fluid and cord blood in relation to prenatal betamethasone load and delivery

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The influence of maternal corticosteroid administration on the cortisol concentration in fetal blood and amniotic fluid (AF) was studied in women receiving betamethasone for prevention of IRDS. Thirty-four pregnant women in danger of spontaneous or induced preterm delivery were treated with 12 mg. of betamethasone daily for 3 days. AF was obtained by amniocentesis on the day before and the day following the betamethasone treatment and by amniotomy at delivery; cord arterial and venous blood was taken at delivery. Corresponding samples were obtained from 17 pregnant control subjects. All samples were analyzed in duplicate for cortisol by radioimmunoassay. The basal AF cortisol level rose with gestational age. The AF cortisol concentration fell from the basal value of 24.5 ± 1.8 to 5.4 ± 0.4 ng. per milliliter 3 days after the start of treatment, and it remained low at delivery if the treatment-delivery time was less than 1 week. An almost significant positive correlation (r = 0.543) was found between the cortisol concentration in cord arterial blood and AF. The cortisol concentration in cord blood in the controls was 108.2 ± 14.3 ng. per milliliter in the arteries and 106.4 ± 18.6 ng. per milliliter in the vein. Also, the cord blood cortisol level was depressed if betamethasone had been given within one week before delivery. The multifactorial influence on cord blood cortisol level prevents interpretation of the results as support for the concept that the human fetal adrenal is involved in labor initiation. The duration of gestation was not altered by the betamethasone treatment. The analysis of cortisol in the easily accessible amniotic fluid is suggested for estimating the function of the fetal adrenal cortex.

CORTICOSTEROIDS seem to play an important role for induction of several biologic mechanisms during fetal life. Glucocorticoids are believed to participate in the initiation of parturition in certain domestic animals such as sheep[1–5] and goats.[6] The lung maturation in utero seems to be influenced by fetal glucocorticoid production,[7] and prenatal administration of glucocorticoids might reduce the incidence of the respiratory distress syndrome in premature infants.[8] Recently, cortisol in cord blood was found to be lower in infants who subsequently developed respiratory distress than in infants without this complication.[9]

In the present investigation, betamethasone was given to pregnant women to prevent respiratory distress of the newborn where there was a risk of induced or spontaneous premature labour. To study any effect on the hormone levels in the fetoplacental unit exerted by the betamethasone treatment, the cortisol concentrations in the amniotic fluid and in the cord blood were monitored in these cases and in a control group. An attempt was also made to evaluate factors influencing the fetal cortisol levels around the time of parturition.

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Material and methods

Thirty-four pregnant women, all in danger of spontaneous or induced preterm delivery, received corticosteroid treatment in a controlled study on the

Table IA. Clinical diagnosis, number of cases

	Betamethasone Group	*Controls*
Imminent premature labor	11	8
History of early premature delivery	4	3
Pre-eclampsia	1	2
Diabetes	—	1
Low urinary estriol and/or retarded fetal growth	7	1
Premature rupture of membranes	7	—
Incompatibility of Rh or ABO system	2	1
Maternal malignancy	1	1
Hepatosis of pregnancy	1	—
Total	34	17

prevention of the respiratory distress syndrome. Seventeen other gravidas in similar risk pregnancies served as controls; they were not given corticosteroid treatment, but apart from that were subjected to the same investigations and treatment as the study group. Seven of the women in the study group had premature rupture of the membranes at the start of the observation period; in all the control subjects, the membranes were intact until the beginning of delivery. All women were hospitalized and were in the last trimester of pregnancy. No cases of polyhydramnios were included in the material. No perinatal deaths occurred and all infants appeared normal at routine clinical examinations. Tables IA and IB give the clinical data of the two groups.

Amniocentesis was usually performed twice on all patients with 3 days' interval, during which the women in the study group were given, as a daily intramuscular injection, 6 mg. of fast-acting betamethasone disodium phosphate and 6 mg. of slow-acting betamethasone acetate (Celestone Chronodose, Schering Corp.). No amniotic taps were performed in the women with ruptured membranes at admittance. At delivery, amniotic fluid was obtained before or after the start of labor by elective amniotomy where there were intact membranes.

As presented in Results, the depressant effect exerted by the betamethasone administration on the cortisol levels at delivery, both in the amniotic fluid and in the cord blood, had completely disappeared 3 weeks after the treatment started. Therefore, the cortisol values where the treatment-delivery interval exceeded 3 weeks were added to the controls in order to enlarge this group for some of the statistical evaluations.

Noncentrifuged amniotic fluid was frozen and stored at −20° C. until analyzed. The umbilical cord was immediately clamped at delivery, and blood in the vein and in the arteries was collected separately in EDTA-containing tubes. Plasma was frozen and stored at −20° C. Cortisol was determined by radioimmunoassay with a commercial kit* according to the manufacturer's recommendations. ^{3}H-labeled cortisol (5,000 c.p.m.) was added to 20 μl of plasma or 100 μl of amniotic fluid to monitor and correct for procedural losses. Each sample was analyzed in duplicate. The coefficient of variation of duplicate determinations in the assay was 3.9 per cent (intraassay) and 8.9 per cent (interassay). The results obtained with this method represent total nonconjugated cortisol. Betamethasone disodium phosphate tested in amounts corresponding to as much as 1,600 ng. per milliliter of plasma did not interfere with the cortisol determination.

Conventional statistical methods were employed. The critical level for rejection of significance was $p = 0.05$.

Results

Amniotic fluid. The cortisol concentration in the amniotic fluid (AF) obtained at the first amniocentesis was of similar over-all magnitude in the betamethasone group (24.5 ± 1.8 ng. per milliliter, n = 18) and the control group (28.2 ± 2.9 ng. per milliliter, n = 17). The short-term variability of the cortisol concentration was low: the intraindividual difference between cortisol concentrations in two amniotic fluid specimens obtained with 3 days' interval in the control group was 3.2 ± 2.0 ng. per milliliter. No sex difference was found in the cortisol concentration in the amniotic fluid; the level was 27.3 ± 2.2 (n = 17) in cases with a female fetus and 25.4 ± 2.6 (n = 18) in cases with a male fetus.

The administration of betamethasone markedly decreased the AF cortisol concentration from 24.5 ± 1.8 ng. per milliliter (n = 18) to 5.4 ± 0.4 ng. per milliliter (n = 19) 3 days after the start of the treatment. At delivery, the AF cortisol concentration remained low if delivery occurred within 1 week after the start of treatment. At longer treatment-delivery intervals, the AF cortisol concentration did not differ from that of the control group (Table II).

As is evident in Table II, in the controls and in the betamethasone group with the treatment-delivery time exceeding 21 days the AF cortisol concentration was significantly higher ($p < 0.05$) at delivery than at the first amniocentesis. When the influence of the gestational age on the AF cortisol concentration was further evaluated in gravidas before labor, the cortisol value after the thirty-sixth week was found to be significantly higher than before this age ($p < 0.01$; Table III). A

*Cortisol ^{125}I-radioimmunoassay kit, Micromedics Systems Inc., Worsham, Pennsylvania 19044.

Table IB. Clinical data (means ± S.E.M.)

Interval, start of study to delivery, (days)	*Gest. age at start of study (wk.)*	*Gest. age at birth (wk.)*	*Apgar score at 1 min.*	*Labor, induced/spont.*	*Delivery, vaginal/cesarean section*	*Duration of labor (hr.)*	*Birth wt. (Gm.)*
Betamethasone-treated:							
≤ 7 (3.3 ± 0.7) n = 7	35.3 ± 0.9	36.0 ± 0.8	7 ± 1	4/3	4/3	6.6 ± 3.2	2206 ± 167
8 – 21 (13.1 ± 1.1) n = 11	33.5 ± 0.5	35.4 ± 0.6	8 ± 0	3/8	7/4	4.6 ± 1.2	2252 ± 170
> 21 (43.3 ± 3.1) n = 16	31.9 ± 0.4	37.9 ± 0.5	8 ± 0	8/8	10/6	4.2 ± 0.9	2703 ± 157
Total betamethasone-treated: n = 34	33.1 ± 0.4	36.7 ± 0.4	8 ± 0	15/19	21/13	4.8 ± 0.8	2454 ± 104
Controls: (29.7 ± 4.3) n = 17	33.4 ± 0.5	37.5 ± 0.5	8 ± 0	6/11	12/5	6.1 ± 1.0	2639 ± 163

weak but significant correlation appeared between the AF cortisol concentration and the gestational age between the thirtieth and fortieth week ($r = 0.378$; $p < 0.01$; Fig. 1).

When the relation between the AF cortisol values at delivery and the mode of initiation of labor was analyzed, two groups of parturients were compared. There was no statistically significant difference of AF cortisol levels between the women with spontaneous onset of labor (n = 10) and those with no labor (five elective sections and six vaginal amniotomies) (Table IV). It is important to note that, in the former group, the amniotic fluid was obtained after an average of 4 hours' labor, whereas in the second group, the samples were taken before the onset of labor. This, if anything, would tend to increase any cortisol difference between the groups (Table IV). The gestational age in the two groups was identical.

Umbilical blood. The cortisol concentration in the umbilical blood at delivery had a somewhat higher value in the arteries than in the vein, both in the betamethasone group (84.4 ± 10.9 and 79.2 ± 10.1 ng. per milliliter, respectively) and in the control group (108.2 ± 14.3 and 106.4 ± 18.6 ng. per milliliter, respectively). The arteriovenous difference, however, was not significant in these groups or in the subgroups discussed further on; therefore only the arterial cortisol levels in the umbilical blood are considered in the following.

The umbilical cortisol level in the group receiving betamethasone treatment was significantly lower than that of the controls when delivery occurred within 7 days after the start of the treatment ($t = 5.633$; $p < 0.001$). There was no significant difference between the treated women and the controls at posttreatment intervals exceeding 7 days (Table V).

Apart from the betamethasone treatment, also the following factors were considered for a possible influence on the cortisol concentration in the cord blood at delivery: (1) the gestational age, (2) the spontaneous onset of labor, and (3) the duration of labor. The amalgamated groups of the controls and the women with betamethasone treatment more than 3 weeks before delivery were used for these evaluations. The cortisol level in umbilical arterial blood rose with the gestational age, as it did in the amniotic fluid (Table VI). The difference between the youngest and the oldest group was significant ($p < 0.05$) but the groups were not homogeneous with respect to the other factors (2), (3). A study of the relation between the duration of labor and the cord arterial cortisol level (Fig. 2) showed that both the group with spontaneous labor and that with induced onset of labor tended to have a reduced cortisol level when the duration of labor diminished. Analysis of the interrelation between the cortisol level, the gestational age, and the duration of labor in groups of different delivery modes showed no statistically significant correlations (Table VII). This is undoubtedly ascribable to the heterogeneity of the clinical material.

The cord blood cortisol in the controls and in the betamethasone group with posttreatment interval exceeding 3 weeks was not significantly correlated to either the the birth weight of the infant or the placental weight ($r = 0.28$ and $r = -0.08$, respectively). Only one woman in the betamethasone group and one in the control group were delivered of a child with Apgar-score at 1 minute below 7. The umbilical cortisol level

Table II. Cortisol in amniotic fluid (ng./ml.)*

Interval, start of study to delivery	*First amniocentesis*	*Second amniocentesis*	*Delivery*
Betamethasone-treated:			
A. ≤7 days	29.4	6.7	9.3
(4.0 ± 1.1)	±8.7	±1.7	±2.4
	(3)	(3)	(4)
		n.s.	n.s.
B. 8-21 days	25.6	5.4	30.7
(15.0 ± 1.5)	±3.8	±0.6	±13.0
	(4)	(5)	(5)
		$p < 0.01$	n.s.
C. >21 days	22.8	5.0	31.1
(43.9 ± 3.2)	±1.5	±0.5	±2.6
	(11)	(11)	(10)
		$p < 0.001$	$p < 0.05$
Total	24.5	5.4	26.4
(28.7 ± 3.9)	±1.8	±0.4	±4.0
	(18)	(19)	(19)
		$p < 0.001$	n.s.
Controls:	28.2	25.6	38.1
(29.7 ± 4.3)	±2.9	±2.2	±2.9
	(17)	(14)	(12)
		n.s.	$p < 0.05$

*Cortisol in amniotic fluid before (first amniocentesis) and after (second amniocentesis) administration of 12 mg. of betamethasone to the woman daily for 3 days, and at delivery. Values of control subjects not receiving betamethasone are given for corresponding times. Significance of difference in table refers to value at first amniocentesis for each group. At delivery the difference between A and the control group was highly significant. The differences between B and the control group and between C and the control group were not significant (means ± S.E.M.). Figures in parentheses denote number of cases.

in both these cases was low, i.e., 50.8 and 69.9 ng. per milliliter, respectively.

The time from the first amniocentesis until *spontaneous* delivery was compared between the controls (n = 11) and the gravida receiving betamethasone (n = 12). Here, all patients with premature rupture of the membranes were excluded because of the obvious risk of coming into premature labor. No significant difference in the postamniocentesis interval was encountered between the two groups (Table VIII). It is important to note that the gestational age at delivery was identical in both groups.

Comment

The study of the pituitary-adrenal axis in the human fetus is attractive for several reasons: (1) the elucidation of basal physiologic mechanisms, i.e., the function of the fetal adrenals in relation to the gestational age, the transfer of adrenal steroids into intrauterine compartments like the amniotic fluid, and the dependence of the fetal adrenals on the fetal pituitary; (2) the search for evidence that the human fetal adrenal is functionally involved in the initiation of labor, as suggested for some other species; (3) evaluation of the role of the adrenal steroids for induction of the synthesis of surface-active phospholipids in the fetal lung.

Table III. Amniotic fluid cortisol concentration (ng./ml.) in relation to gestational age*

Gestational age (wk.)	
< 36	*≥ 36*
25.2	33.4
±1.4	±2.6
(43)	(17)

*$t = 2.7926$; $p < 0.01$. The figures are calculated on results from amniocentesis on 35 untreated gravidas (49 samples) and from amniotomy at delivery before induced labor on 11 parturients: untreated (5 samples) or with treatment—delivery time exceeding 21 days (6 samples) (mean ± S.E.M.).

Table IV. Amniotic fluid cortisol at delivery in cases without or with spontaneous onset of labor*

	Amniotic fluid cortisol (ng./ml.)	*Gestational age (wk.)*	*Duration of labor (hr.)*
No labor	33.5	37.5	—
(n = 11)	±3.4	±0.6	
Spontaneous labor	38.8	37.9	4.3
(n = 10)	±3.2	±0.8	±1.2
Significance of difference	$t = 1.1464$ n.s.		

*Amniotic fluid obtained at cesarean section or amniotomy in both groups (mean ± S.E.M.).

Our knowledge of the hormonal state of the human fetus is largely based on measurements of hormonal concentrations in umbilical blood after delivery; extrapolations from these values backward to the time before birth seem to be less valid. For technical reasons, it has not been feasible to obtain blood safely from the fetus in utero. Amniotic fluid, on the other hand, is readily available from diagnostic amniocenteses. In the present work, advantage was taken of the amnion taps made for measuring the effect on the fetal surfactants of an inductive treatment with betamethasone.[10] Thus, we followed the influence of a large betamethasone dose admistered to the pregnant woman on the cortisol concentration in the amniotic fluid and in the umbilical blood at various times after the administration. The betamethasone effect on the phospholipids in the amniotic fluid is excluded from the present report; it will be published elsewhere.[10]

The *cortisol concentration* in human *amniotic fluid* at delivery found in the present study agrees with corresponding values obtained by fluorometric determination[11] and by a competitive protein binding

Table V. Cortisol in cord blood (ng./ml.)*

Interval, start of study to delivery	*Umbilical artery*	*Umbilical vein*
Betamethasone-treated:		
≤ 7 days	24.7	25.2
(3.3 ± 0.7)	±3.9	±3.1
	(6)	(6)
	p < 0.001	p < 0.001
8-21 days	86.3	76.4
(12.9 ± 1.2)	±20.2	±15.4
	(10)	(10)
	n.s.	n.s.
> 21 days	107.2	102.7
(42.7 ± 3.2)	±14.5	±15.0
	(15)	(15)
	n.s.	n.s.
Controls:	108.2	106.4
(27.8 ± 5.4)	±14.3	±18.6
	(12)	(12)

*Cortisol in arterial and venous cord blood at delivery at various intervals after administration of 12 mg. of betamethasone daily for 3 days to the pregnant woman, and in control subjects (mean ± S.E.M.). Number of cases in parentheses. Significance of difference in regard to controls.

Table VI. Cortisol in arterial cord blood (ng./mg.)*

	Gestational age at delivery (wk.)		
	≤ 36	*37-39*	*≥ 40*
	73.3	115.9	120.6
	±11.5	±15.8	±17.6
	(6)	(14)	(7)
Significance of difference			
t		2.185	2.248
p		< 0.05	< 0.05

*Cortisol in arterial cord blood in cases without betamethasone treatment or with treatment-delivery time exceeding 21 days (mean ± S.E.M.). Number of cases in parentheses. Significance of difference to youngest group.

method.[12] The rise of the amniotic fluid cortisol concentration with advancing gestational age is paralleled by the observation of an amnion cortisol level at premature delivery lower than at term.[12] The origin of the cortisol in amniotic fluid is not definitely determined. There is strong evidence that, during the last trimester, the fetal urine is a major contributor to the amniotic fluid.[13, 14] It has been shown that in human beings most of the maternal cortisol passing the placenta is converted to cortisone,[15] and it was calculated that the fetus near term produces about 75 per cent of its cortisol.[16] It therefore seems reasonable to infer that the cortisol which reaches the amniotic fluid by the fetal urine is predominantly of fetal origin. If the metabolic clearance rate of amniotic cortisol remains constant and the passage of maternal cortisol through the amniotic membranes is negligible, the cortisol concentration in amniotic fluid can be considered to reflect the integral level of cortisol in fetal plasma for a certain time. This possibility is strengthened by the fact that, in the present investigation,

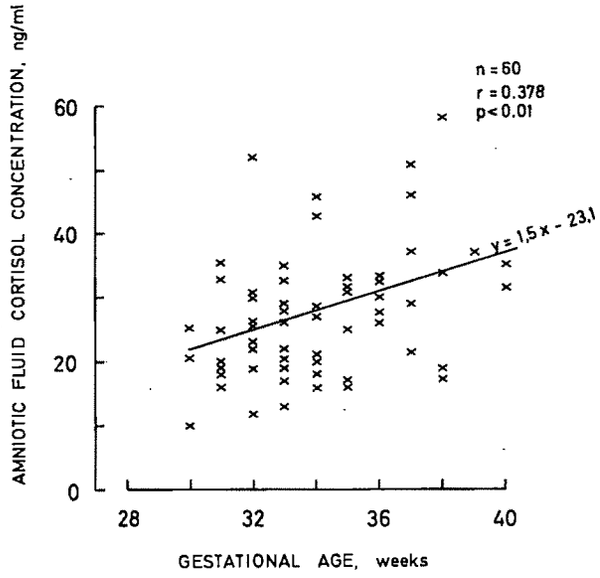


Fig. 1. Correlation between the cortisol concentration in amniotic fluid and the gestational age in 60 samples from 46 women. r = 0.378; p < 0.01. The same samples as in Table III.

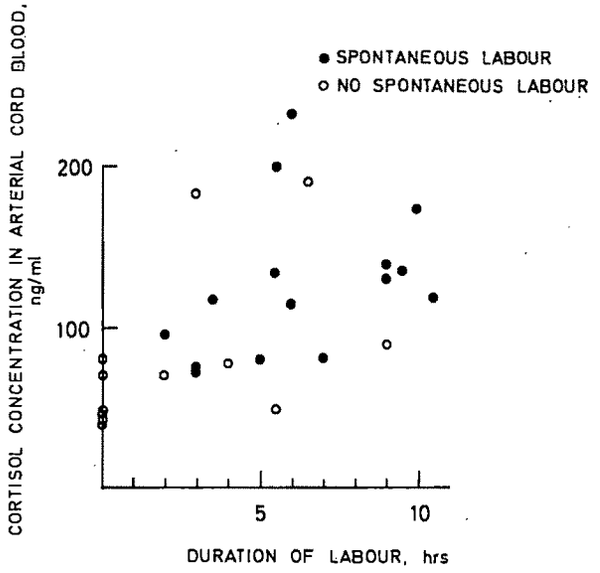


Fig. 2. The relation between the cortisol concentration in arterial cord blood and the duration of labor in 15 cases with spontaneous labor (filled symbols) and 12 cases of elective sections and induced labor (open symbols).

Table VII. Correlation between cortisol concentration in umbilical blood and gestational age and duration of labor, respectively, after different modes of delivery

Elective cesarean section (n = 6)		*Induced labor (n = 7)*		*Spontaneous labor (n = 14)*	
Cortisol in umbilical cord	*Gest. age*	*Gest. age*	*Duration of labor*	*Gest. age*	*Duration of labor*
Artery	r = 0.685 n.s.	r = 0.093 n.s.	r = 0.269 n.s.	r = 0.156 n.s.	r = 0.303 n.s.
Vein	r = 0.427 n.s.	r = −0.004 n.s.	r = 0.419 n.s.	r = 0.263 n.s.	r = 0.310 n.s.

Table VIII. Comparison of the interval from first amniocentesis to spontaneous delivery in the betamethasone group and the control subjects*

Betamethasone group (n = 12)			*Control subjects (n = 11)*		
Gest. age at first amniocentesis (wk.)	*Gest. age at delivery (wk.)*	*Interval (days)*	*Gest. age at first amniocentesis (wk.)*	*Gest. age at delivery (wk.)*	*Interval (days)*
32.1 ±0.3	37.3 ±0.6	36.1 ±6.1	32.8 ±0.6	37.3 ±0.8	32.1 ±5.7
n.s.	n.s.	n.s.			

*Cases with premature rupture of membranes were excluded (mean ± S.E.M.).

betamethasone administration occasioned a quantitatively identical reduction of cortisol in the umbilical artery blood (to 22.8 per cent of controls) and in the amniotic fluid (to 22.0 per cent of original level) 3 days after the start of the treatment. It was earlier demonstrated that this treatment decreased the maternal plasma cortisol level to 10 per cent of basal value at the same time (3 days after the start of treatment.[17] The changes of the cortisol levels in the amniotic fluid thus correspond more to the fetal than to the maternal blood level changes.

The cortisol concentration in the arterial cord blood compared with that in the amniotic fluid in *simultaneously* obtained samples showed an almost significant positive correlation (r = 0.543). The ratio between the cortisol concentrations in the two compartments was approximately 2:1. This concentration gradient might be explained by different levels of cortisol-binding globulin and albumin in the amniotic fluid and in the cord blood. In fact, the concentration of total protein in the amniotic fluid is 27 times lower than in fetal serum;[18] albumin occupies 60 to 69 per cent of this in amniotic fluid at term[19, 20] vs. 56 per cent in fetal serum.[18] The cortisol binding capacity is lower in fetal than in maternal human plasma,[21] but no reports are available about whether cortisol-binding globulin is present in amniotic fluid.

The static and dynamic relation between the fetal and the amniotic cortisol suggests that a fairly rapid exchange of this steroid occurs between the two compartments. These results point to the possibility of using cortisol analysis in the easily accessible amniotic fluid for estimating the secretion of the fetal adrenals.

Earlier reports on the *cortisol concentration in cord blood* vary considerably, partly owing to different methods of determination, which in some cases might include steroids other than cortisol.[12, 29, 30–32, 34, 35, 41] The wide range of basal cortisol values given might also be ascribed to the fact that cortisol in man is known to be released episodically rather than continuously,[22] and to circadian variations of plasma cortisol concentration which are present in pregnant women[23] and which might influence the cord blood levels too. The present results, however, agree with other reported levels when only the competitive protein-binding method used at cesarean section is considered.

The concept that the fetal pituitary-adrenal axis plays an important role in the initiation of labor is based on observations and experiments mainly on sheep.[1, 2, 4, 24, 25] The influence of the human fetal adrenal function on the onset of parturition has been more difficult to study and has been suggested by the alleged prolongation of pregnancy in some cases of anencephaly.[26] On the other hand, in several other

forms of congenital adrenal hypoplasia in man, the gestational period has been of normal duration.[27, 28] It has been repeatedly observed that the concentration of corticosteroids in cord blood is higher at vaginal deliveries than at cesarean sections.[29–32] The cortisol levels in cord blood have been determined in several recent studies and related to various modes of delivery, giving inconsistent results. A lower level of cord blood cortisol was found at elective cesarean sections compared with indicated sections[33] and vaginal deliveries[34]; equal cortisol levels in these groups have also been reported.[35] This inconsistency seems to be partly ascribable to the fact that these studies have not conclusively distinguished between the alleged influence on plasma cortisol by factors such as the spontaneous or induced initiation of parturition, the labor stress, and the gestational age. As is suggested by the present investigation, some of these factors, such as the duration of labor and the stage of gestation, seem to determine the cord blood cortisol concentration at delivery. The influence on the fetal cortisol level exerted by the stress in utero[36, 12] and by the gestational age[23] has, in fact, been shown recently. Therefore, due to the multifactorial regulation of cord blood cortisol level, the present results cannot answer the question whether in the human fetus a rise in plasma cortisol precedes the initiation of spontaneous labor.

Hitherto, there has thus been little definite support for the concept that, in man and in the nonhuman primates, the fetal adrenals are involved in the labor initiation, in contrast to what has been found in sheep. This discrepancy seems logical in view of the fact that the human placenta is not impermeable to cortisol,[16, 37] which means that the fetal plasma cortisol level is to some extent influenced by the maternal level.[38] The present study clearly shows that large doses of betamethasone to the mother did not reduce the gestational period. This suggests that betamethasone cannot replace cortisol in any alleged role for labor initiation. A similar conclusion regarding dexamethasone has recently been reached in both man[39] and rhesus monkey.[40] On the other hand, the induced decrease of the blood cortisol concentration in the fetus did not postpone the parturition. Whether this lack of effect on the duration of gestation is due to the transient nature of the fetal cortisol depression in the present study remains to be elucidated.

In a preliminary investigation using a fluorometric assay, a significant reduction of the cortisol concentration in the amniotic fluid was observed after treatment of pregnant women with betamethasone.[17] This finding was substantiated in the present work by means of a radioimmunoassay. The effect of betamethasone on the amniotic fluid cortisol paralleled that of cord blood in time course and magnitude, and is consistent with a presumed passage of betamethasone into the fetoplacental unit where it exerts a negative feedback function on the fetal pituitary. This presumption is validated by a recent study where maternally administered cortisol was found to pass the placenta and cause a 57 per cent diminution of ACTH in umbilical artery plasma.[41] There is now substantial evidence that the human fetus contributes to its own plasma cortisol level.[33, 34] The present investigation clearly shows that, with pharmacologic doses of betamethasone, it is possible to lower the level of endogenous cortisol in fetal plasma. Further study is necessary to determine whether such treatment would turn off a prematurely activated mechanisms for labor induction.

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Serum levels of a placental protein during gestation in the baboon

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A placental specific protein (Sp-1) isolated from human placentas was used to establish a radioimmunoassay for measurement of a baboon protein in the sera of pregnant baboons. No reaction was observed with any known pituitary or placental hormone in this assay system. Levels of Sp-1 were detected at 17 to 18 days of gestation and reached high levels by the end of the first trimester of pregnancy. A slow increase in serum levels continued thereafter until parturition. Disappearance of Sp-1 post partum was very slow and required 26 to 34 days to become undetectable in the serum. No function or role of Sp-1 in pregnancy has been suggested, but possible use of its measurement for detecting abnormal placental function deserves investigation.

SEVERAL proteins have been isolated from placental tissue and have been shown to be present in the serum of pregnant women. Among these are hormones, including human chorionic gonadotropin (HCG), human chorionic somatomammotropin (HCS), and human chorionic thyrotropin (HTS). Recently, one of us (H. B.) has demonstrated the existence of placental proteins other than those listed above that are believed to be produced solely from placental tissue.[1] One of these proteins, herein referred to as Sp-1, is also present in the maternal serum in large amounts during pregnancy.

Preliminary biochemical characterization of Sp-1 has been performed,[2, 3] although no known hormonal or other biologic function of this substance has been identified. Antisera to Sp-1 have been prepared and a specific radioimmunoassay for it has been established. Extracts from baboon placentas and serum from pregnant baboons react very strongly in this assay system for human Sp-1. This report deals with the measurement of a baboon Sp-1–like protein in the serum of six pregnant baboons throughout gestation as a preliminary study to the role and function of this substance in pregnancy.

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Materials and methods

Animals. Female baboons *(Papio cynocephalus)* were housed and maintained as previously described.[4] Pregnancy was established by housing females with a male of proved fertility for 3 days at midcycle, beginning 2 days prior to expected ovulation. Pregnancy determinations and the course of gestation were monitored by hormone determinations in serial serum samples collected at frequent intervals after mating. Blood samples were collected more frequently at the beginning and end of gestation and during the postpartum period. Serum samples were stored frozen (−20° C.) until assayed for hormone and Sp-1 content.

Assays. Measurements of baboon chorionic gonadotropic (CG) levels were performed by the method reported earlier.[4] Similar procedures were used to assay serum levels of baboon chorionic somatomammotropin (CS). A highly purified preparation of baboon CS[5] was used to prepare antisera in rabbits, to prepare ^{125}I-labeled tracer, and for use as a standard in the baboon CS radioimmunoassay. Antisera did not react with other baboon or human pituitary or placenta hormones excepting human CS. Likewise, ^{125}I-labeled baboon CS did not bind to antisera raised to human and baboon hormones other than anti-HCS. The sensitivity of the assay was 10 ng. per milliliter and

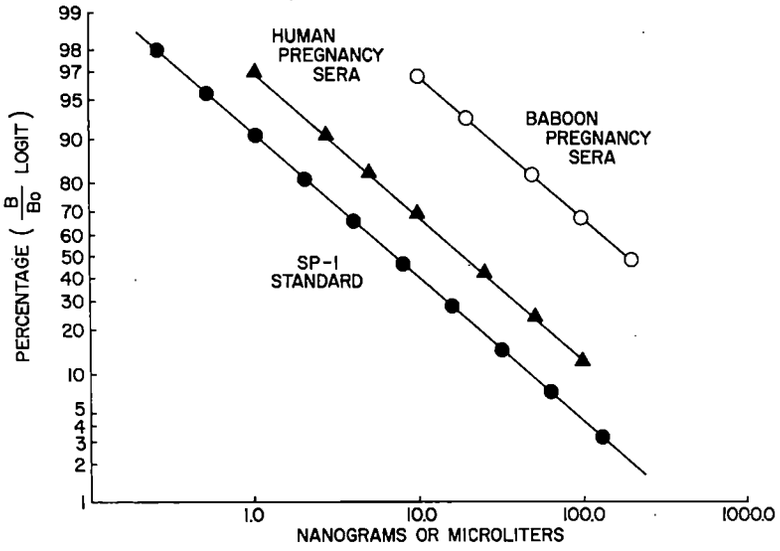


Fig. 1. Dose response lines to standard and pregnancy serum in the Sp-1 radioimmunoassay.

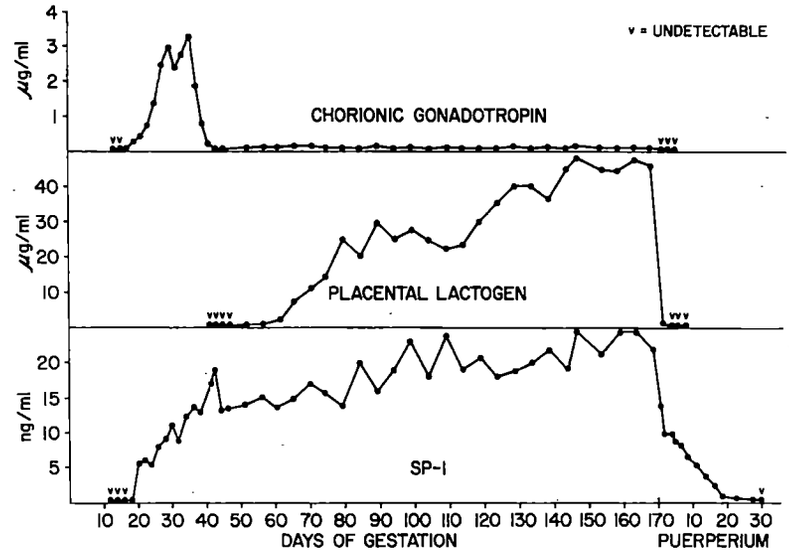


Fig. 2. Patterns of serum Sp-1, baboon chorionic gonadotropin, and baboon placental lactogen during a normal baboon pregnancy. Parturition in this animal was at 170 days of gestation.

the over-all precision of this system permitted reproducibility of serum values within ±12 per cent.

A radioimmunoassay for Sp-1 was developed with essentially identical procedures used for other protein hormones. Highly purified Sp-1 was radiolabeled with ^{125}I according to a modified procedure of Greenwood, Hunter, and Glover.[6] Iodinated Sp-1 (250 pg.) with a specific activity of 95 to 125 μc per microgram was reacted with 200 μl of rabbit antisera to Sp-1 in a final dilution of 1:160,000. This serum dilution bound approximately 112 pg. of the labeled antigen and was used for most assays reported here. Sheep antirabbit gamma globulin (200 μl) was employed in this double-antibody radioimmunoassay system.

No binding of anti-Sp-1 serum in dilutions from 1:1,000 to 1:100,000 was observed to radiolabeled human HCG, HCS, FSH, LH, TSH, HGH, beta subunit of HCG, or baboon CG, LH, and CS. Likewise, no binding of 250 pg. of ^{125}I Sp-1 was observed with high-teter antisera to human FSH, LH, HCG, HCS, HGH, TSH, beta subunit of HCG, or baboon CG, LH, and CS. Thus, no evidence was found to indicate nonspecific reactions of this assay to any known substances in pregnant baboon sera.

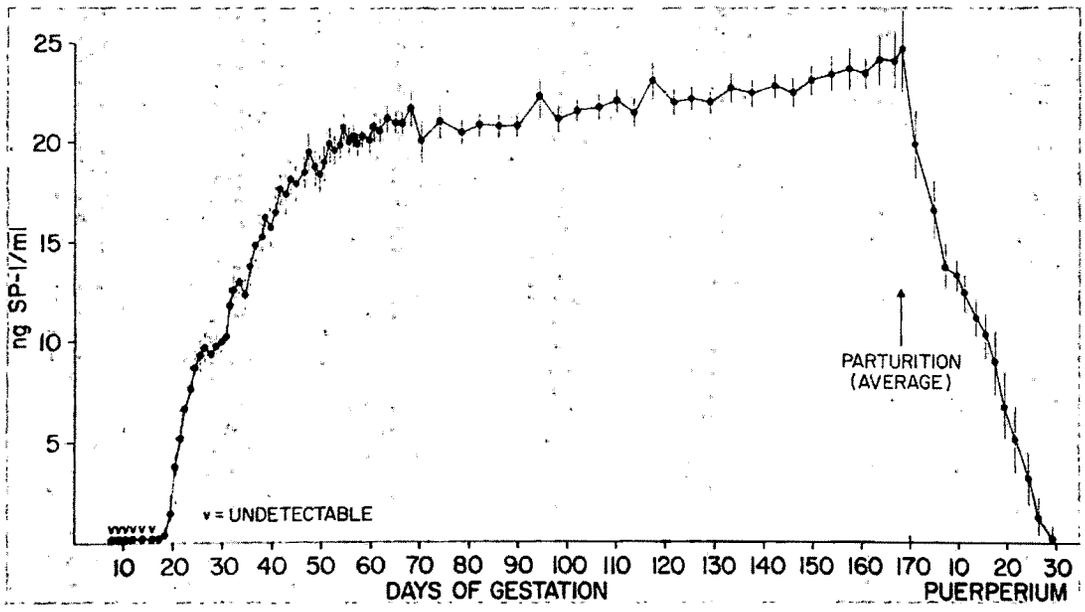


Fig. 3. Mean serum Sp-1 levels from six normal baboon pregnancies. Date of parturition indicated was the mean for all animals. Vertical lines represent the standard error.

Assay buffers, incubation procedures, and statistical analyses of data used have been described previously.[4] A typical dose-response line in an Sp-1 assay is shown in Fig. 1. The minimum detectable amount of Sp-1 in this system was 1.5 ng. per milliliter and the mean intra-assay coefficient of variation within 22 assays was 3.5 per cent. Also illustrated in Fig. 1 is the obvious parallelism in responses of Sp-1, human pregnancy sera, and baboon pregnancy sera. This observation was statistically confirmed ($P < 0.001$).

Varying amounts of sera were assayed depending upon the stage of gestation when samples were collected (20 to 200 μl). Assay sensitivity was intentionally reduced for the second- and third-trimester samples to increase assay precision. All values were reported as nanograms per milliliter equivalents of highly purified human Sp-1 since the same material used for radiolabeling was used for assay standard.

Results

Patterns of Sp-1 levels during pregnancy in the baboon were very consistent among the six animals studied. Sp-1 was detected on day 17 or 18 in each animal, although pregnancy dating could be in error ±1 day. Levels rose rapidly from day 20 to day 50 of gestation to about 18 ng. per milliliter, then increased very slowly thereafter to a level of approximately 24 ng. per milliliter prior to parturition. Following delivery of the infant, Sp-1 levels declined very slowly and undetectable levels were not reached until 26 to 34 days post partum in the six baboons.

The slight variation observed in Sp-1 patterns was characterized by a flat plateau of levels from day 65 until term in one female and a slight decline in levels prior to parturition in another. Maximum levels and rate of increase during early gestation was quantitatively virtually identical in all animals. Rates of postpartum disappearance were also remarkably similar. A pattern of Sp-1 levels during pregnancy for an individual animal is illustrated in Fig. 2 together with patterns of baboon chorionic gonadotropin and chorionic somatomammotropin for comparison. A composite pattern of mean Sp-1 levels for all six animals is shown in Fig. 3.

Comment

Specific antibodies to Sp-1 raised in rabbits do not react immunologically with pregnancy sera from rat, dog, rabbit, mouse, hamster, or guinea pig,[7] but do react with sera from rhesus monkeys, cynomolgus monkeys, and baboons. The rabbit anti-Sp-1 sera have also been shown to produce abortions in pregnant cynomolgus monkeys passively immunized between days 19 and 55 of gestation.[8] Thus, it appears that this substance is secreted by the placentas of several primate species and is important to the maintenance of pregnancy.

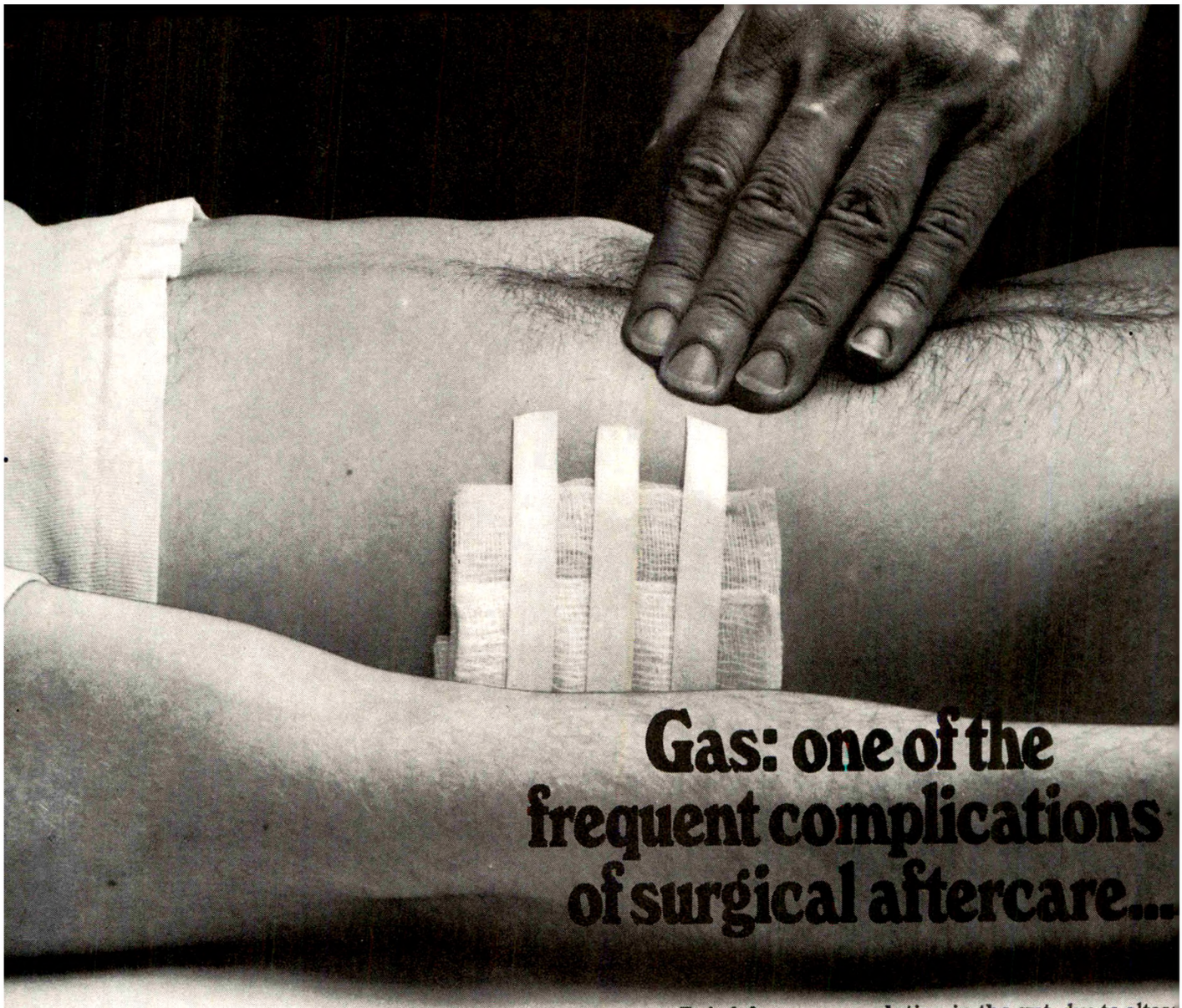
Measurements of Sp-1 by radial immunodiffusion tests in human serum at various weeks of pregnancy have been reported.[1, 9] Levels of Sp-1 around 2.0 mg. per milliliter were detected during the twentieth week of human gestation and continued to rise slowly until

around the thirty-sixth week, when a plateau was retained until term. This pattern is distinctly different from that of the baboon, which reached plateau levels by the end of the first trimester of pregnancy. In addition, the concentration of Sp-1 in human pregnancy sera was one- to 5,000-fold higher than in baboons. Differences in the assay methodology used may account for the differences in time of Sp-1 detection and the large differences in quantitative levels attained during gestation, but the difference in species patterns cannot be ascribed to measurement procedures.

Levels of Sp-1 in the sera of women experiencing problem pregnancies appear worthy of investigation. Since levels of this substance are very high during most of gestation, significant changes in the level of Sp-1 may be associated with alterations in placental structure or function. Depending upon the variation in serum levels in normal pregnancies, such changes in Sp-1 levels may be useful in the diagnosis of abnormal placental function. Further, the observation that specific antibodies to Sp-1 will terminate normal pregnancy in monkeys suggests that this protein may be useful in the development of methods of human fertility control.

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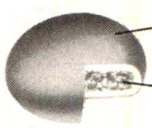
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Painful gas accumulation in the gut, due to altere intestinal motility, aerophagia and increased salivation, often complicates surgical aftercare.

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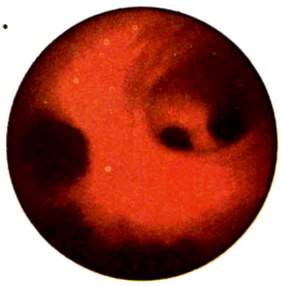
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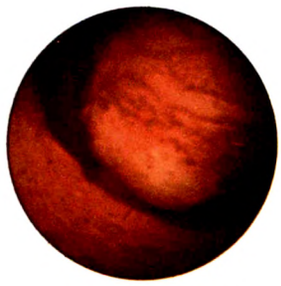
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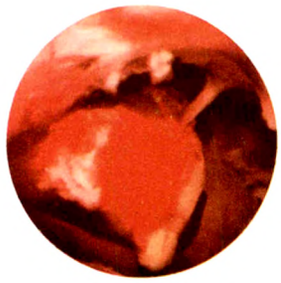
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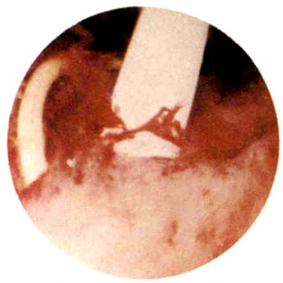
Asherman's Syndrome



a submucous fibroid



an endometrial polyp



an embedded IUD

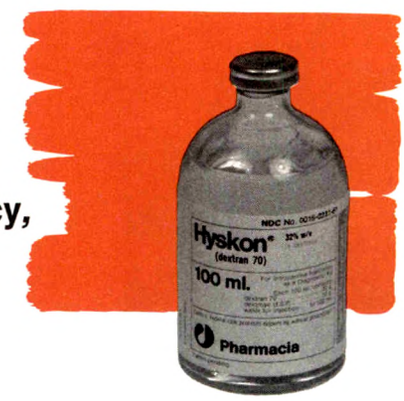
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(32% dextran 70)

HYSTEROSCOPY FLUID–

an intrauterine distending solution that now...

- provides a crystal clear hysteroscopic view of intrauterine surfaces;
- permits ample time for directly visualized treatment with no interference from blood or mucus;[1,7]
- adds a new dimension of direct visualization, accuracy, and ease in the detection and treatment of intrauterine problems.[1-8]



For additional information on Hysteroscopy with **HYSKON®**
contact: Medical Director, Pharmacia Laboratories, Piscataway, N.J. 08854

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Pharmacia

PHARMACIA LABORATORIES INC.
PISCATAWAY, NEW JERSEY 08854

PRESCRIBING INFORMATION

HYSKON® HYSTEROSCOPY FLUID

32% (W/V) dextran 70 in dextrose

DESCRIPTION: Hyskon Hysteroscopy Fluid is a clear, viscid, sterile, non-pyrogenic solution of dextran 70 (32% W/V) in dextrose (10% W/V). Dextran 70 is that fraction of dextran, a branched polysaccharide composed of glucose units, having a weight average molecular weight of 70,000. The fluid is electrolyte-free and non-conductive. At room temperature Hyskon Hysteroscopy Fluid has a viscosity of 220 cSt.

Hyskon has a tendency to crystallize when subjected to temperature variations or when stored for long periods. If flakes of dextran are present, heat at 100°-110° C until complete dissolution is achieved.

INDICATIONS: Hyskon Hysteroscopy Fluid is indicated for use with the hysteroscope as an aid in distending the uterine cavity and in irrigating and visualizing its surfaces.

CONTRAINDICATIONS: Hyskon Hysteroscopy Fluid should not be instilled in patients known to be hypersensitive to dextran. All other contraindications are those relative to the hysteroscopic procedure itself, such as pregnancy, endometrial carcinoma, etc.

WARNINGS: It is not known whether systemic absorption of dextran 70 occurs from the uterine and peritoneal cavities. Because of the possibility that absorption can occur, the physician should familiarize himself with the systemic effects of this drug. The most alarming are severe, sometimes fatal anaphylactoid reactions, which are quite rare. Other noteworthy effects include plasma volume expansion and transient prolongation of bleeding time.

ADVERSE REACTION: Undesirable effects directly attributable to the use of Hyskon Hysteroscopy Fluid have not been reported. However, the physician should be mindful of the fact that adverse reactions are known to occur with the intravenous administration of dextran 70 (MACRODEX 6% W/V). Such reactions have included: allergic phenomena (anaphylactoid reactions, urticaria, nasal congestion, wheezing, tightness of chest and mild hypotension), nausea, vomiting, fever and joint pains.

DOSAGE AND ADMINISTRATION: The amount of Hyskon Hysteroscopy Fluid required per patient depends on a number of factors, such as the type and length of the diagnostic procedure, whether or not manipulation or surgery is performed, etc. Most often, however, the amount of Hyskon instilled into the uterus will be between 50 and 100 milliliters.

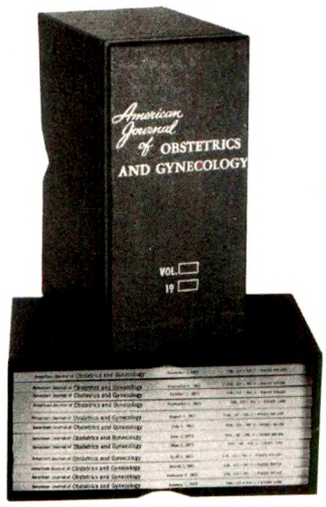
Hyskon should be introduced into the uterine cavity through the cannula of a hysteroscope under low pressure (approximately 100 mm Hg) until the uterus is sufficiently distended to permit adequate visualization. During the hysteroscopic examination, Hyskon should be infused at a rate that keeps the cavity suitably distended. To avoid injection of the fluid into the tissues of the uterus and parametria and to avoid having unnecessary amounts of the fluid pass into the peritoneal cavity and backwards along the side of the hysteroscope, infusion pressures greater than 150 mm Hg should not be used.

HOW SUPPLIED: Hyskon Hysteroscopy Fluid (32% W/V dextran 70 in 10% W/V dextrose) is available as a sterile, nonpyrogenic solution in 100 ml bottles packed 12 to a carton.
NDC No. 0016-0231-61
Keep from cold during transportation.

CAUTION: Federal law prohibits dispensing without prescription.

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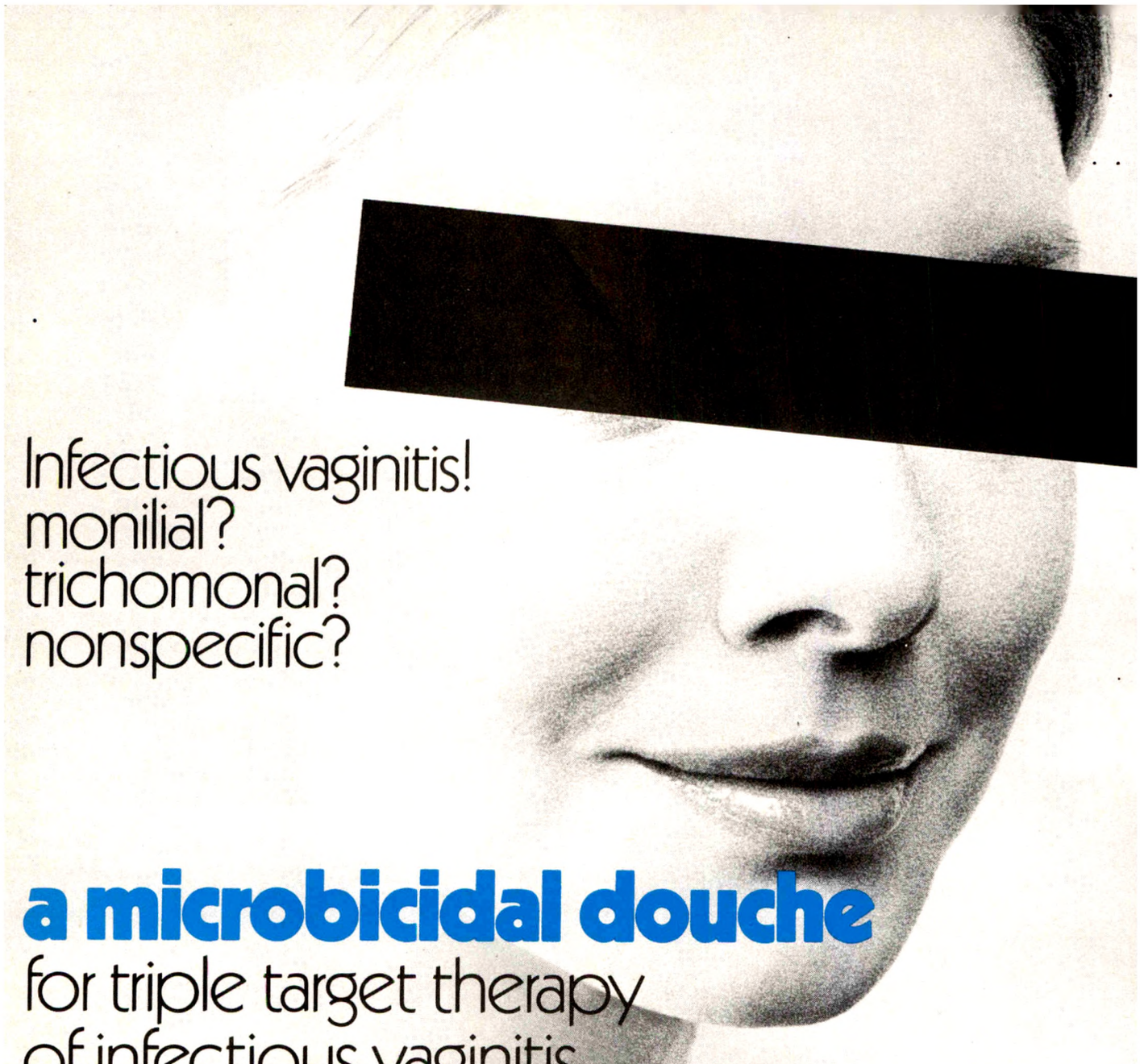
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monilial?
trichomonal?
nonspecific?

a microbicidal douche

for triple target therapy
of infectious vaginitis

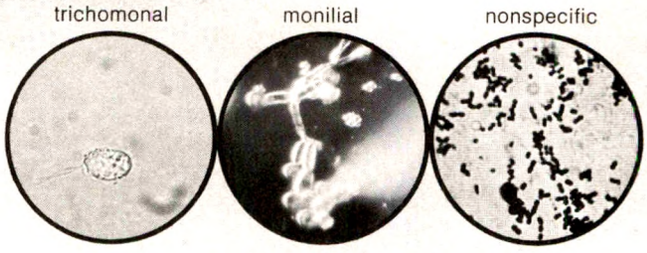
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Infectious vaginitis often involves a mixed infection. Systemic or topical therapy directed against a single pathogen may miss or encourage another.

BETADINE DOUCHE is a broad-spectrum topical microbicide that promptly kills trichomonads, monilia and bacteria. It is active in the presence of blood, pus or vaginal secretions, and is virtually nonirritating to vaginal mucosa. Low surface tension assists penetration into vaginal crypts and crevices.

Suggested regimen for therapeutic use: In the office, swab the cervix and vulvovaginal area with BETADINE SOLUTION. Prescribe BETADINE DOUCHE: Two (2) tablespoonfuls to a quart of lukewarm water once daily, preferably before bedtime, through the entire menstrual cycle. If further therapy is warranted, douching may be continued through the next cycle. The patient may be instructed to return for an office visit following two weeks of therapy.

Directions for routine cleansing: One (1) tablespoonful to a quart of lukewarm water once or twice a week.



Supplied: 8 oz. and 1 gal. plastic bottles; 1/2 oz. packettes (6 per carton).

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A microbicidal douche packaged for patient convenience

BETADINE® douche

for triple target therapy of infectious vaginitis (trichomonal, monilial, nonspecific)...

for regular home use in therapy and routine cleansing

BETADINE douche 8 oz.

8 oz. plastic bottle with measuring cap for proper dosage



for continuing therapy or cleansing when traveling—or when convenient, single-use packages are preferred

BETADINE douche packette ½ oz.

½ oz. premeasured dose packettes (6 per carton)

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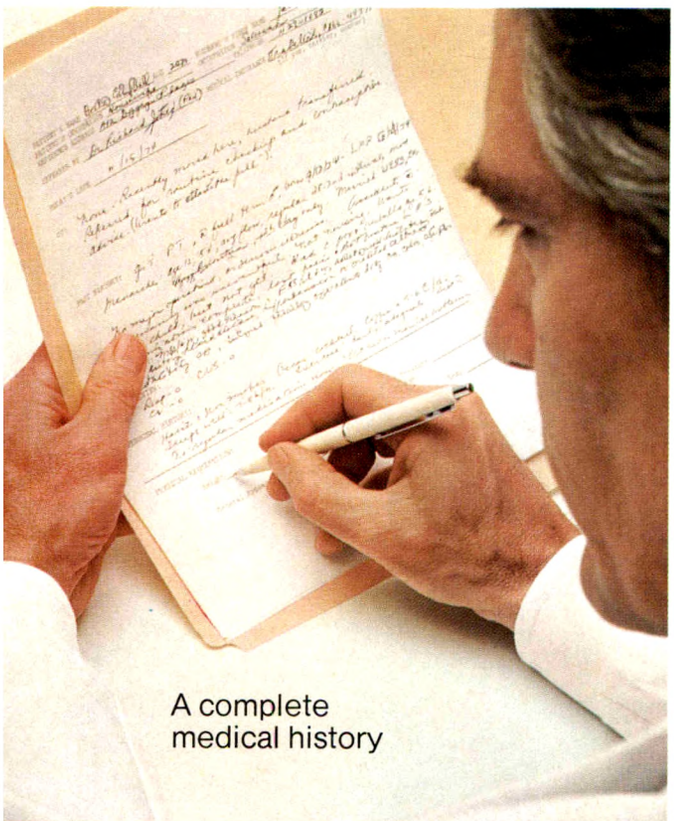
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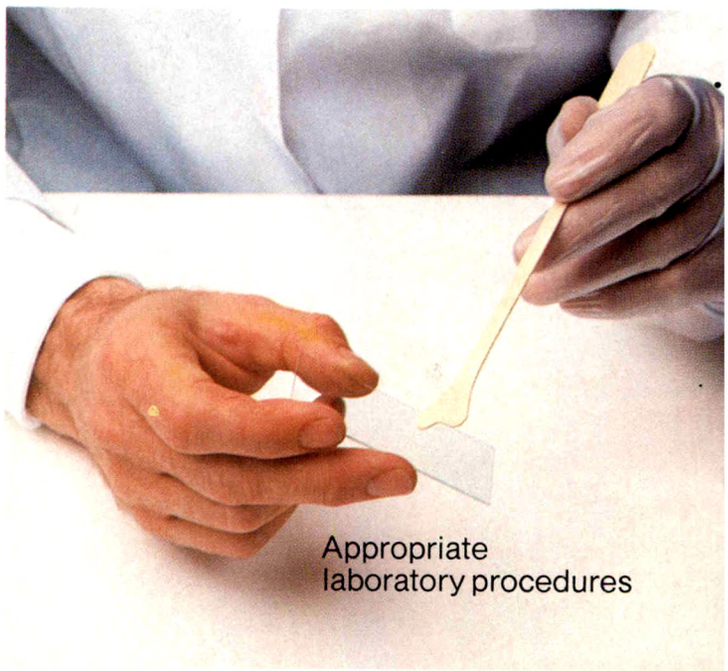
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Prudent Practice...

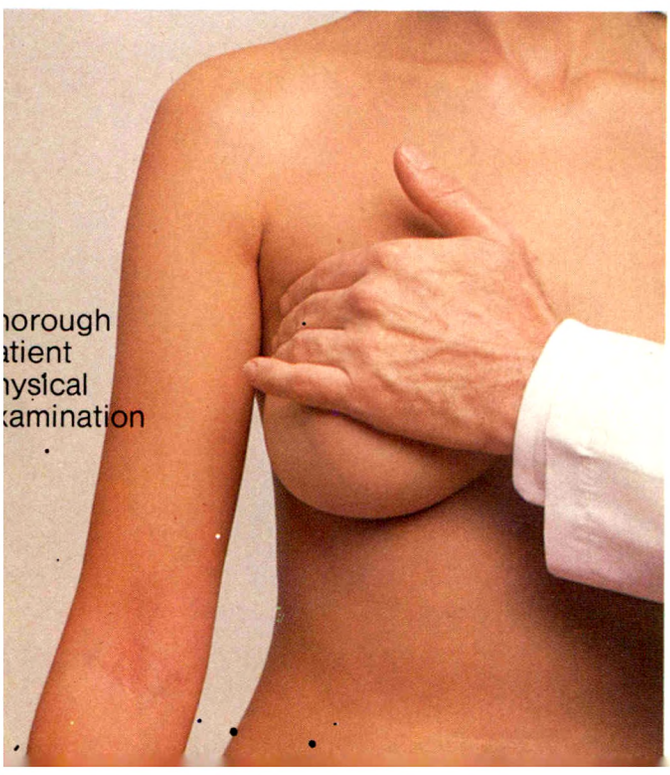
Some sensible steps physicians take before prescribing an oral contraceptive



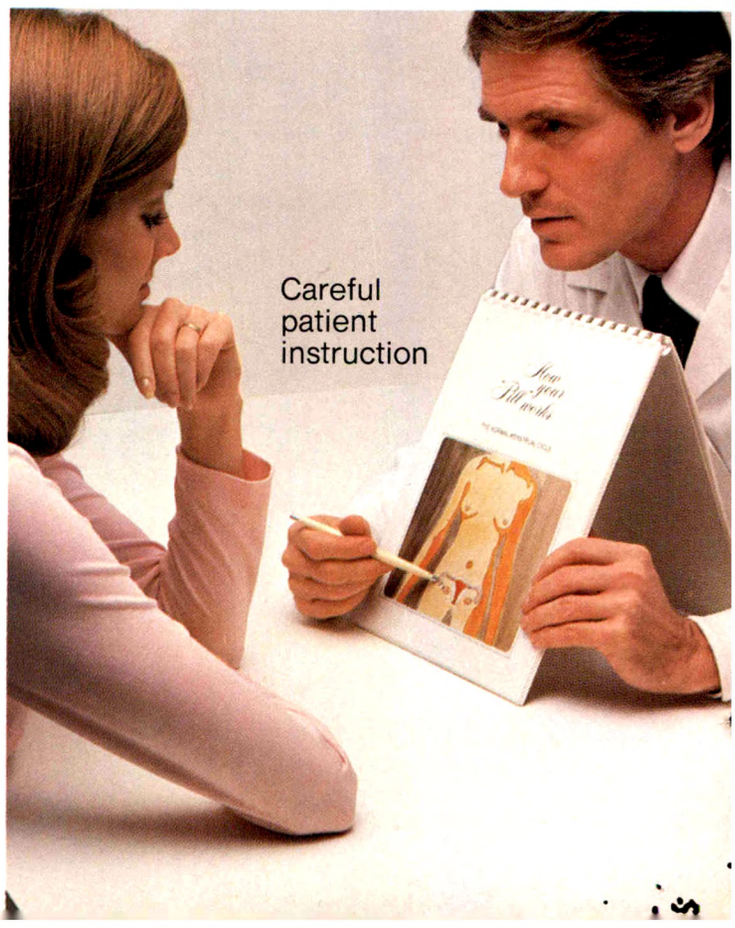
A complete medical history



Appropriate laboratory procedures



horough
atient
hysical
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Careful patient instruction

And when you prescribe a combination oral contraceptive, it's also prudent practice to employ the lowest effective dose of estrogen that is otherwise acceptable.

For many women, this could mean Loestrin 1/20—20 mcg of ethinyl estradiol in combination with 1 mg of norethindrone acetate.

As you would expect, irregular bleeding and occasionally the absence of bleeding, especially during the first few cycles, are more frequent with Loestrin 1/20 than with combinations containing larger doses of estrogen. Nevertheless, clinical trials demonstrated that more than 85% of the subjects, some of whom experienced such irregular cycles, found Loestrin 1/20 an acceptable oral contraceptive.

Effective contraception... with a low dose of estrogen—a tough combination to beat.

The lowest dose of a proven effective estrogen in a combination oral contraceptive

LOESTRIN® 1/20

Each white tablet contains 1 mg norethindrone acetate and 20 mcg ethinyl estradiol; each brown tablet contains 75 mg ferrous fumarate, USP.

PARKE-DAVIS



See next page for prescribing information.

LOESTRIN 1/20

Each white tablet contains:
norethindrone acetate (17 alpha-ethinyl-19-nortestosterone acetate) . . . 1 mg
ethinyl estradiol (17 alpha-ethinyl-1,3,5 (10)-estratriene-3, 17 beta-diol) . . . . . 20 mcg
Each brown tablet contains
75 mg ferrous fumarate, USP

BRIEF SUMMARY OF
PRESCRIBING INFORMATION

SPECIAL NOTE

Oral contraceptives have been marketed in the United States since 1960. Reported pregnancy rates vary from product to product. The effectiveness of Loestrin 1/20 is somewhat lower than that of the higher-dose combination products. Both provide almost completely effective contraception.

The dropout rate for medical reasons, as observed in the clinical trials that were conducted with Loestrin 1/20, appears to be somewhat higher than observed with higher-dose combination products. This higher dropout rate was observed to be due particularly to irregular bleeding.

An increased risk of thromboembolic disease associated with the use of hormonal contraceptives has now been shown in studies conducted in both Great Britain and the United States. Other risks, such as those of elevated blood pressure, liver disease, and reduced tolerance to carbohydrates, have not been quantitated with precision.

Long-term administration of both natural and synthetic estrogens in subprimate animal species in multiples of the human dose increases the frequency for some animal carcinomas. These data cannot be transposed directly to man. The possible carcinogenicity due to the estrogens can neither be affirmed nor refuted at this time. Close clinical surveillance of all women taking oral contraceptives must be continued.

INDICATION

Loestrin 1/20 is indicated for the control of conception.

CONTRAINDICATIONS

1. Thrombophlebitis, thromboembolic disorders, cerebral apoplexy, or a past history of these conditions.
2. Markedly impaired liver function.
3. Known or suspected carcinoma of the breast.
4. Known or suspected estrogen-dependent neoplasia.
5. Undiagnosed abnormal genital bleeding.
6. Pregnancy.

WARNINGS

1. The physician should be alert to the earliest manifestations of thrombotic disorders (thrombophlebitis, cerebrovascular disorders, pulmonary embolism, and retinal thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately.

Retrospective studies of morbidity and mortality in Great Britain and studies of morbidity in the United States have shown a statistically significant association between thrombophlebitis, pulmonary embolism, and cerebral thrombosis and embolism and the use of oral contraceptives. There have been three principal studies in Britain [1-3] leading to this conclusion, and one[4] in this country. The estimate of the relative risk of thromboembolism in the study by Vessey and Doll[3] was about sevenfold, while Sartwell and associates in the United States found a relative risk of 4.4, meaning that the users are several times as likely to undergo thromboembolic disease without evident cause as nonusers. The American study also indicated that the risk did not persist after discontinuation of administration, and that it was not enhanced by long continued administration. The American study was not designed to evaluate a difference between products. However, the study suggested that there might be an increased risk of thromboembolic disease in users of sequential products. This risk cannot be quantitated, and further studies to confirm this finding are desirable.

2. Discontinue medication pending examination if there is sudden partial or complete loss of vision, or if there is a sudden onset of proptosis, diplopia, or migraine. If examination reveals papilledema or retinal vascular lesions, medication should be withdrawn.

3. Since the safety of Loestrin 1/20 in pregnancy has not been demonstrated, it is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period.

4. A small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The long-range effect to the nursing infant cannot be determined at this time.

PRECAUTIONS

1. The pretreatment and periodic physical examinations should include special reference to breasts and pelvic organs, including Papanicolaou smear, since estrogens have been known to produce tumors, some of them malignant, in five species of subprimate animals.

2. Endocrine and possibly liver function tests may be affected by treatment with Loestrin 1/20. Therefore, if such tests are abnormal in a patient taking Loestrin 1/20, it is recommended that they be repeated after the drug has been withdrawn for two months.

3. Under the influence of estrogen-progestogen preparations, preexisting uterine fibromyomata may increase in size.

4. Because these agents may cause some degree of fluid retention, conditions which might be influenced by this factor, such as epilepsy, migraine, asthma, cardiac or renal dysfunction, require careful observation.

5. An alteration in menstrual patterns in many patients is likely to be induced by using Loestrin 1/20 The amount and duration of flow and cycle length may be variable. Because of these irregular bleeding patterns, it is important that the physician take this into consideration when attempting to determine if persistent bleeding is from organic causes.

6. Patients with a history of psychic depression should be carefully observed and the drug discontinued if the depression recurs to a serious degree.

7. Any possible influence of prolonged Loestrin 1/20 therapy on pituitary, ovarian, adrenal, hepatic, or uterine function awaits further study.

8. A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. The mechanism of this decrease is obscure. For this reason, diabetic patients should be carefully observed while receiving Loestrin 1/20 therapy.

9. The age of the patient constitutes no absolute limiting factor, although treatment with Loestrin 1/20 may mask the onset of the climacteric.

10. The pathologist should be advised of Loestrin 1/20 therapy when relevant specimens are submitted.

11. Susceptible women may experience an increase in blood pressure following administration of contraceptive steroids.

ADVERSE REACTIONS OBSERVED IN PATIENTS RECEIVING ORAL CONTRACEPTIVES

A statistically significant association has been demonstrated between use of oral contraceptives and the following serious adverse reactions:

Thrombophlebitis
Cerebral thrombosis
Pulmonary embolism

Although available evidence is suggestive of an association, such a relationship has been neither confirmed nor refuted for the following serious adverse reactions:

Neuro-ocular lesions, e.g, retinal thrombosis and optic neuritis

The following adverse reactions are known to occur in patients receiving oral contraceptives:

Nausea
Vomiting
Gastrointestinal symptoms (such as abdominal cramps and bloating)
Breakthrough bleeding
Spotting
Change in menstrual flow
Amenorrhea during and after treatment
Edema
Chloasma or melasma
Breast changes: tenderness, enlargement, and secretion
Change in weight (increase or decrease)
Changes in cervical erosion and cervical secretions
Suppression of lactation when given immediately postpartum
Cholestatic jaundice
Migraine
Rash (Allergic)
Rise in blood pressure in susceptible individuals
Mental depression

Although the following adverse reactions have been reported in users of oral contraceptives, an association has been neither confirmed nor refuted:

Anovulation post treatment
Premenstrual-like syndrome
Changes in libido
Changes in appetite
Cystitis-like syndrome
Headache
Nervousness
Dizziness
Fatigue
Backache
Hirsutism
Loss of scalp hair
Erythema multiforme
Erythema nodosum
Hemorrhagic eruption
Itching

The following laboratory results may be altered by the use of oral contraceptives:

Hepatic function: increased sulfobromophthalein retention and other tests

Coagulation tests: increase in prothrombin, factors VII, VIII, IX, and X.

Thyroid function: increase in PBI and butanol extractable protein-bound iodine, and decrease in T^3 uptake values

Metyrapone test

Pregnanediol determination

DOSAGE AND ADMINISTRATION

Special Notes on Administration

Menstruation usually begins two or three days, but may begin as late as the fourth or fifth day, after the brown tablets have been started. Because of the relatively low estrogenic content, Loestrin 1/20 is not a good cyclic regulator. There are patients whose inherent hormone balance will require larger amounts of estrogen to achieve cyclic regularity than that contained in Loestrin 1/20. These patients experience altered bleeding patterns, which do not conform to treatment schedules, while taking the white Loestrin 1/20 tablets. However, it is important that patients adhere to the dosage schedule regardless of when bleeding occurs.

The occurrence of altered bleeding is highest in Cycle 1. The majority of patients will have seven days or less of total bleeding during the 28-day cycle (including both withdrawal bleeding and breakthrough bleeding and spotting). Should irregular bleeding occur, patients should be reassured and instructed to continue taking the tablets as directed. If by the third cycle the irregular patterns are unacceptable, consideration should be given to changing the medication to a product with higher estrogen content (Norlestrin®-1 mg or Norlestrin-2.5 mg). The physician should be alert to the fact that the irregular bleeding patterns could mask bleeding from organic causes, and appropriate diagnostic measures should be taken if the bleeding persists or continues after changing to a higher estrogen-content product.

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GYNECOLOGY

Pure gonadal dysgenesis with an XY chromosomal constitution (Swyer's syndrome): Report of two cases

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Two cases of Swyer's syndrome, characterized by rudimentary streaks in association with a 46,XY chromosome karyotype are reported. Both individuals were tall, with sparse axillary and pubic hair, and breasts were undeveloped in one and well developed in the other (she had some estrogen therapy). One had infantile but otherwise normal external genitals, while the other had poor development of labia majora and no clitoris. The vagina in each was normal, with a small cervix and uterus, and vaginal smears of the preadolescent type. Urinary 17-ketosteroids were normal, while gonadotropin levels were elevated. The gonadal streaks were extirpated, and histologic examination revealed the presence of fibrous stroma but no ova or follicles. Scattered clumps of Leydig cells and mesonephric remnants were found in one patient. Both patients responded well to cyclic hormonal therapy, i.e., menstrual withdrawal bleeding and breast development.

GONADAL DYSGENESIS, stricto sensu, applies to a primary congenital gonadal defect due to failure of differentiation of the gonad before its full sexualization. The prototype of gonadal dysgenesis is the multidystrophic syndrome of Turner with its extragenital somatic anomalies as dominant features of the disorder. There is a possibility of normal genital function associated with dystrophic states (similar to those encountered in Turner's syndrome) as well as the existence of gonadal dysgenesis without them. The presence of chromosomal aberrations serves to confuse the situation. Even though 45,X sex chromosome compliment seems to be the most frequent single pattern, there are other karyotypes including chromosome mosaicism and those with normal sex chromosome patterns, male or female.

The XY sex chromosome compliment may be encountered in isolation or in mosaicism. We previously reported five cases of gonadal dysgenesis with XO/XY mosaicism[1]; four of these were assymetric gonadal dysgenesis with a gonadal streak on one side and an immature testis on the other. The normal male sex chromosome pattern, XY, may be encountered in three different types of gonadal dysgenesis: gonadal dysgenesis with gonocytoma, male Turner's syndrome, and so-called pure gonadal dysgenesis with which this article is concerned.

In 1955, Swyer[2] described two chromatin-negative

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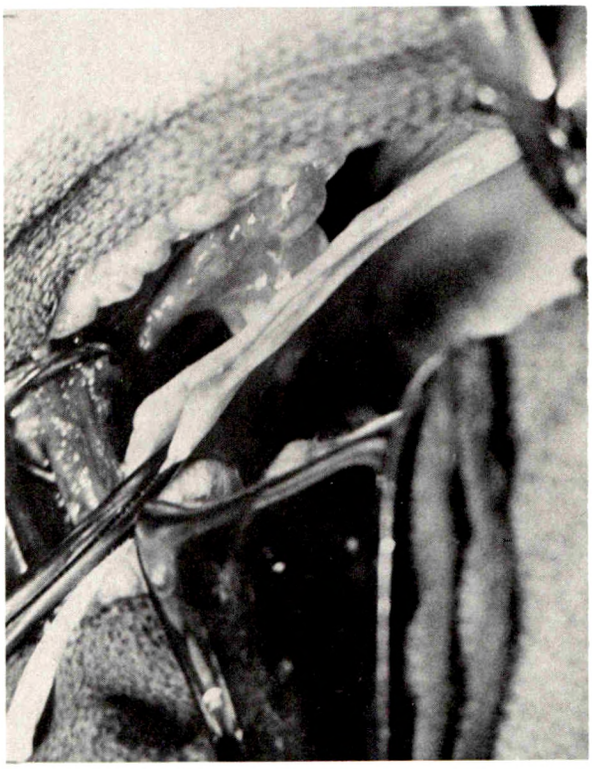


Fig. 1. Case 1. surgical findings, showing rudimentary gonadal streaks.

girls who were of tall stature and lacked the anomalies and malformations usually encountered in Turner's syndrome. Hoffenberg and Jackson[3] suggested that the cases described by Swyer were a variant of gonadal dysgenesis. In 1959, Harnden and Stewart[4] found a normal male chromosome compliment in a tall 19-year-old girl with primary amenorrhea, and they named the disorder "pure gonadal dysgenesis." Since then, this appellation has been used for patients with a variety of karyotypes, provided they did not have the somatic anomalies usually found in Turner's syndrome. If the chromosomal compliment 45,X is never found in pure gonadal dysgenesis, there may be justification for separating those subjects who have a female phenotype and XY sex chromosome complex (Swyer's syndrome) from those with the other karyotypes encountered, including various mosaics and a normal female XX chromosomal constitution. It is believed that this entity is an intrauterine castration syndrome in a subject who is genetically male. It mimics Jost's[5] castration experiment in rabbits, a condition different from those in which the gonad appears destined to form an ovary.[6]

Case reports

Case 1. L. H. was a 17-year-old girl with primary amenorrhea and failure of sexual development.



Fig. 2. Case 1. Photomocrograph showing mesonephric remnant.

Height was 166 cm. Positive physical findings included sparse axillary and pubic hair, undeveloped breasts, and infantile but otherwise normal external genitals. On pelvic examination, a tight virginal introitus precluded visualization of the cervix, but no masses were palpable rectally. Radiologic examination of hands and wrists revealed all epiphyses to be open. Urinary 17-ketosteroids were 6.9 and 7.5 mg. per 24 hours. Urinary follicle-stimulating hormone (FSH) determinations were greater than 52 and less than 104 mU. per 24 hours. Vaginal smears were preadolescent in type, and the nuclear sex chromatin pattern was negative. Chromosome counts from leukocyte cultures consistently showed 46 chromosomes, and analysis revealed an XY sex chromosome complex no different from that of a normal male subject. At laparotomy, typical gonadal streaks were found (Fig. 1). The uterus was represented by a small rudimentary structure. Microscopically, the gonadal streaks showed a small amount of fibrous stroma, scattered clumps of Leydig cells, mesonephric remnants, but no ova or follicles (Figs. 2 and 3).

Case 2. A. B. was a 25-year-old female subject with primary amenorrhea who failed to menstruate following various hormonal regimens. Height was 170 cm. Sexual hair pattern was typical of an adult female but

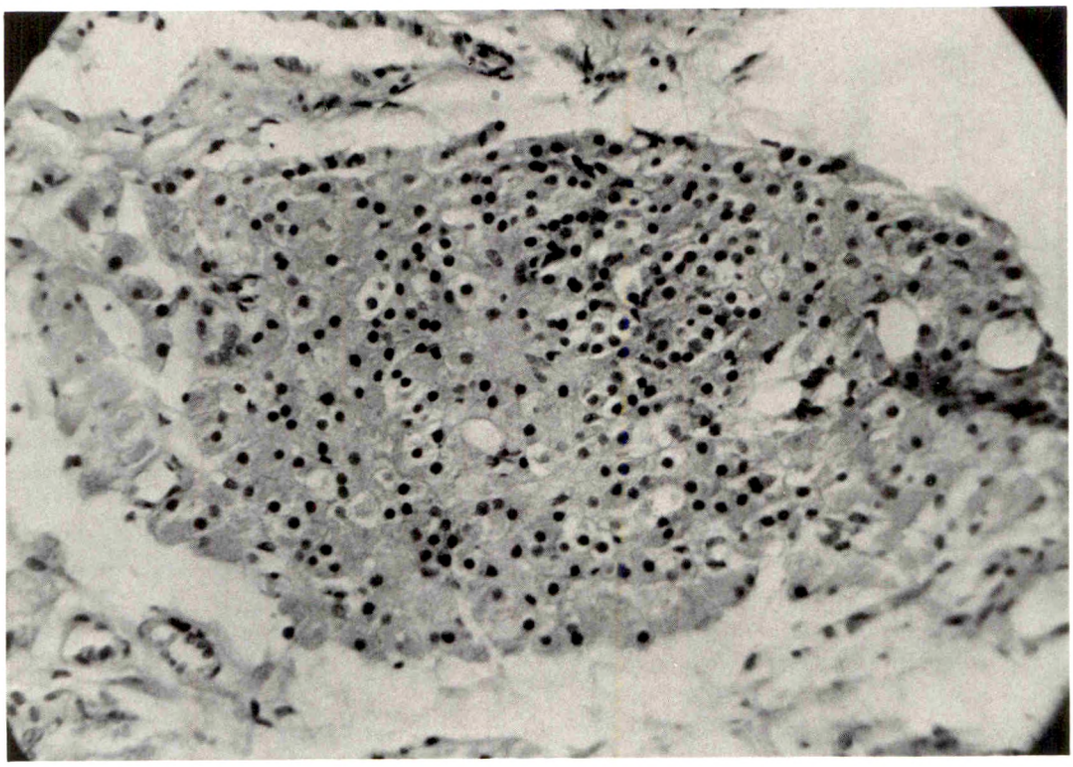


Fig. 3. Case 1. Photomicrograph showing typical clump of Leydig cells.

quantitatively poor, and breasts were well developed (she had received some estrogen therapy in the past). The external genitals showed an absence of development of the labia majora and an almost nonexistant clitoris. Pelvic examination revealed a normal vagina and small cervix and uterus with nonpalpable adnexa. Endocrine studies were performed, and a castrate vaginal smear was obtained. 17-Ketosteroids were 8 and 9.6 mg. per 24 hours. Plasma gonadotropin levels obtained by radioimmunoassay were: FSH, 43.7 mI.U. per milliliter, and LH, 4.5 mI.U. per milliliter.

Gynecography combined with a hysterosalpingogram showed the existence of a small uterine cavity of normal configuration with Fallopian tubes of normal caliber. There was no clearly defined ovarian density. Sex chromatin was negative, and chromosome analysis from a leukocyte culture demonstrated a 46,XY karyotype (Fig. 4). A laparotomy was performed; the uterus was infantile, and bilateral gonadal streaks were present. Both Fallopian tubes and streaks were removed. Histologic study of the gonadal streaks revealed dense cellular connective tissue, and neither primary oocytes nor Leydig cells were present in the sections examined.

Comment

The classical cases of gonadal dysgenesis (Turner's syndrome) are characterized by amenorrhea, diminished stature, webbed neck, increased carrying angle of arms, low hairline, a "shield" chest, and a 45,X chromosome karyotype. In some exceptional subjects with very well-developed breasts, normal axillary and pubic hair, normal menstruation, and even pregnancy have been reported.[7, 8]

In 1955, when Swyer[2] reported two cases of pure gonadal dysgenesis with a 46,XY karyotpye, he found several common features. Both subjects were tall with little or no mammary development. Axillary and pubic hair distribution, the vagina, and the cervix were within normal limits. The uterus was small, and the vaginal cytology was hypoestrogenic. Urinary output of 17-ketosteroids was normal. The two subjects differed in that in one there was an enlarged clitoris.

In our cases, histopathologic examination of the gonadal streaks revealed the presence of fibrous stroma but no ova or follicles. Scattered clumps of Leydig cells and mesonephric remnants were found in one case. Kinch and associates[9] also reported the same histopathologic findings with the exception of a lack of Leydig cells.

Fibrous tissue is the major component of the gonadal streak.[1, 10] It is not clear that germ cell migration is necessary for gonadal development. In pure gonadal dysgenesis with an XY sex chromosome pattern, normal migration of germ cells may not occur. If this does occur, then the environment probably proves

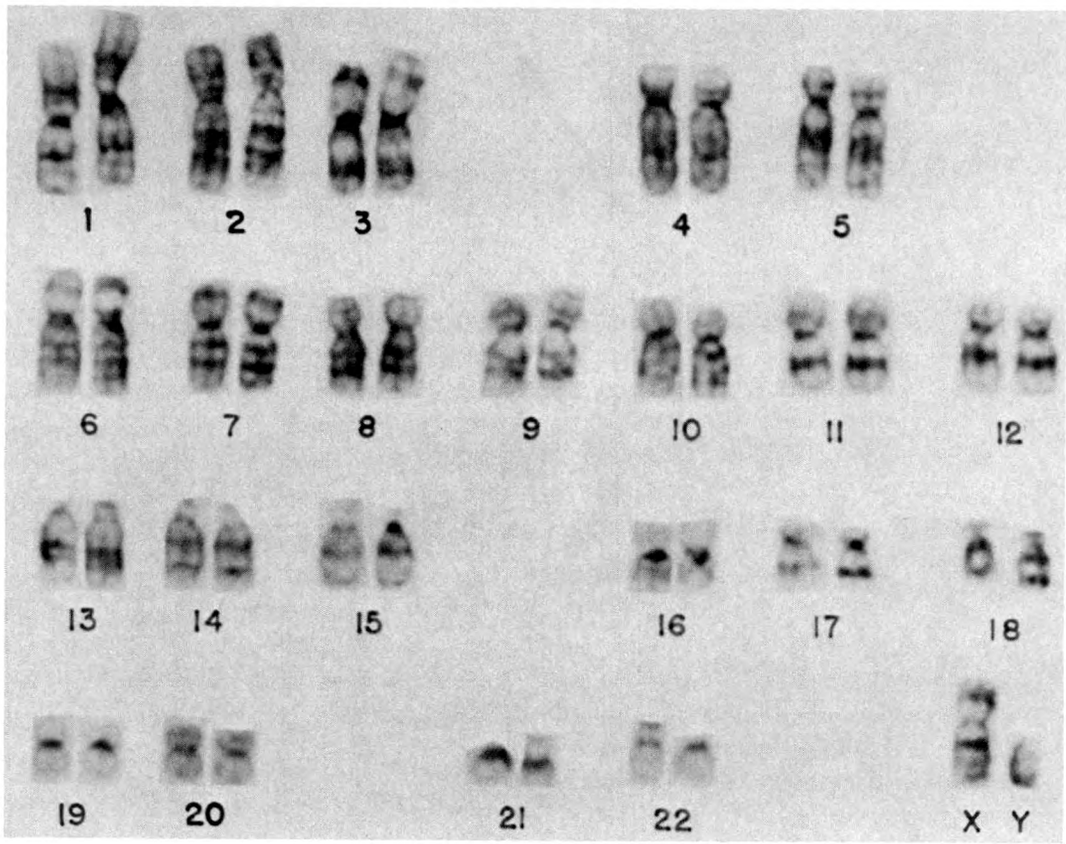


Fig. 4. Case 2. Karyotype showing 46,XY. G-banded.

unsatisfactory for normal development. The belief that gonadal dysgenesis has a genetic etiology is fairly well accepted while the specific genetic mechanism remains unknown. If it is related to multiple genes, the specific alleles and their loci have not been detected.[9, 11, 12] Whatever the cause, the destined testicular development fails to occur early enough in fetal life so that internal and external genitals develop along female lines.[11]

In pure gonadal dysgenesis, one third of the subjects have a 46,XX karyotype.[12] Primary ovarian failure may be due to some early embryonic accident not of chromosomal origin. Two thirds apparently have the 46,XY karyotype, though some of these may be 46,XX and the apparent Y is really the remaining short arm of an X.[12] Libido and breast development may be due to end-organ sensitivity to extragonadal estrogens.

It is recommended in cases of Swyer's syndrome, as well as in other cases of gonadal dysgenesis with an XY cell line, that the streaks be extirpated because of the risk of germ cell tumor development.[13]

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Testosterone metabolism in endometrial cancer

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Plasma testosterone (PC^T), metabolic clearance (MCR^T), and production rates (PR^T) of testosterone and conversion ratio of testosterone to androstenedione (CR^{TA}_{pp}) were performed on 11 patients with endometrial cancer and on a control group of five postmenopausal women. The PC^T, MCR^T, and PR^T of patients with endometrial cancer are in the normal postmenopausal range. The conversion of testosterone to androstenedione in patients with endometrial cancer was significantly decreased.

EPIDEMIOLOGIC studies suggest that estrogens may play an etiologic role in the development of malignancy in women, particularly in breast and endometrial cancer.[1–3] Recent studies have also indicated alterations in androgen metabolism in breast and endometrial cancer.[4, 5] Bulbrook and associates[6–8] found that a discriminant function can be derived from a study of the urinary excretion patterns of 17-ketosteroids and 17-hydroxycorticoids. Patients with a positive discriminant, i.e., those with a high etiocholanolone and/or androsterone excretion relative to the 17-hydroxycorticoids, tended to benefit from adrenalectomy with oophorectomy or hypophysectomy, whereas those with a negative discriminant did not respond. De Waard and associates[9] applied Bulbrook's concept of a hormonal discriminant function to patients with endometrial cancer and have shown that those patients excreted less etiocholanolone relative to 17-hydroxycorticoids or estrogens (mainly estriol) than normal controls.

Moreover, the polycystic ovary syndrome (PCOS), characterized by anovulation and sclerocystic ovaries, is associated with a reported 20 per cent incidence of endometrial cancer.[10–12] This condition is associated with increased plasma levels, production and metabolic clearance of testosterone, as well as elevated gonadotropins.[13, 14]

Sall and Calanog[15] evaluated the urinary steroid excretion patterns of postmenopausal women with benign and neoplastic endometrium and corroborated the findings of low androsterone and etiocholanolone excretions in women with endometrial hyperplasia and cancer. The introduction of precise methods for measuring plasma levels and production rates of the various androgens and their conversions to the estrogens has provided a unique opportunity to determine whether a distinct abnormality in androgen metabolism is indeed present in endometrial cancer.

Our study in endometrial cancer patients encompassed three areas: (1) the evaluation of testosterone metabolism, (2) the evaluation of androstenedione metabolism and its conversions to estrogens, and (3) the evaluation of estrogen metabolism. This initial report concerns itself with the testosterone levels, metabolic clearance rates, production rates, and interconversions to androstenedione of patients with endometrial cancer.

Materials and methods

Eleven patients (two premenopausal and nine postmenopausal) with abnormal uterine bleeding were hospitalized at the New York Medical College and the diagnosis of endometrial cancer was made by fractional

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Table I. Clinical summary of endometrial cancer patients

Patient	*Age*	*Ht. (in.)*	*Wt. (lbs.)*	*Stage of disease*	*Pre-existing medical problem*	*Years menop.*	*Body area ($M.^2$)*
L.M.	46	65	180	I	Hypertension, diabetes	Premenop.	1.89
B.F.	54	60	110	I	Hypertension	5	1.45
J.G.	58	67	230	I	Obesity, hypertension, diabetes	10	2.14
M.F.	45	61	135	I	None	Premenop.	1.60
B.S.	55	60	88	I	Hypertension, diabetes	10	1.31
R.S.	60	62	198	II	Hypertension, obesity	11	1.95
E.J.	60	64	190	II	Obesity, diabetes, hypertension	11	1.92
J.F.	69	61	142	II	Hypertension	19	1.63
E.S.	78	64	230	II	Obesity, hypertension	30	2.08
L.C.	61	64	195	II	Obesity, hypertension	5	1.94
A.W.	78	65	125	III	Hypertension	30	1.62

Table II. Clinical summary of postmenopausal controls

Control	*Age*	*Ht. (in.)*	*Wt. (lbs.)*	*Coexisting medical disease*	*Years post-meno*	*Body area ($M.^2$)*
V.C.	56	62	186	None	7	1.86
I.P.	53	64	195	None	4	1.92
M.Y.	51	61	128	None	4	1.58
D.D.	62	61	165	None	14	1.70
J.C.	55	64	148	None	10	1.64

curettage. A group of five postmenopausal patients served as controls. Clinical summaries of both groups are in Tables I and II.

All patients were hospitalized under basal conditions. The pertinent history and physical examination were obtained. The metabolic clearance (MCR) and production (PR) rates of testosterone and conversion of testosterone to androstenedione were determined with a constant infusion procedure.[13] After an overnight fast the patients were kept in a supine position for at least 1 hour before the start until the end of the constant infusion. The plasma concentration of free testosterone was measured by the protein displacement method of Mayes and Nugent.[16]

In the constant infusion procedure, a priming dose of either 4 or 6 μCi of 1,2^{3}H-testosterone (sp. gr. 137 μCi/μg and found to be 97 per cent pure by paper chromatography in a modified Bush B 3 System) was first administered intravenously as a single injection. Thirty minutes later, an intravenous infusion was started containing either 8 or 12 μCi of 1,2^{3}H-testosterone dissolved in 50 ml. of 8 per cent ethanol in saline. The labeled steroid was given at a constant rate with the aid of a Harvard pump (Model No. 600/900). Then 40 ml. of blood were withdrawn at 105, 120, and 135 minutes after initial injection. The plasma was separated, added to tubes containing 4-^{14}C-testosterone and processed. The value of the radioactivity in each plasma sample present specifically as testosterone and corrected for losses during the isolation procedure was calculated. The mean of the three estimates and the value for the rate of the radioactive infusion (R) were taken to calculate the metabolic clearance rate (MCR) according to the following expression: MCR (L./day) = r (mμCi/day)/Ci(mμCi/L.).

The production rate (PR) of testosterone was then calculated from the expression (PR [mg./day] = MCR [L./day] × MPC [mg./L.]), where MPC is the mean plasma concentration of testosterone.

Results

The kinetic parameters of testosterone metabolism in normal postmenopausal women and in patients with endometrial cancer are given in Tables III and IV, respectively.

Plasma testosterone (PC^T). The mean PC^T (0.60 ± 0.34 [S.D.] μg/L.) in the endometrial cancer group was compared to the mean PC^T (0.40 ± 0.20 [S.D.] μg/L.) of the normal postmenopausal patient group and to a group of 14 normal premenopausal women (0.54 ± 0.11 [S.D.] μg/L.) previously evaluated in our laboratory.[17] There was no significant difference between the mean PC^T in the endometrial cancer group and the normal premenopausal and postmenopausal groups.

Metabolic clearance rates of testosterone (MCR^T). The mean MCR^T (735 ± 180 [S.D.] L./day) in the

Table III. Plasma concentrations, metabolic clearances, production rates, and interconversions of testosterone in postmenopausal controls

Patient	PC^T *(μg/L.)*	MCR^T *(L./day)*	PR^T *(μg/day)*	CR^{TA}_{pp}
J.C.	0.38	698	558	0.040
I.P.	0.76	823	625	0.042
M.Y.	0.28	714	200	0.043
D.D.	0.30	721	216	0.039
J.C.	0.29	695	202	0.041
Mean ± (S.D.)	0.402 ± 0.20	730 ± 53	364 ± 218	0.042 ± 0

Table IV. Plasma concentrations, metabolic clearances, production rates and interconversions of testosterone in endometrial cancer patients

Patient	*Stage of disease*	PC^T *(μg/L.)*	MCR^T *(L./day)*	PR^T *(μg/day)*	CR^{TA}_{pp}
L.M.	I	1.04	667	694	0.020
B.F.	I	0.62	594	368	0.018
J.G.	I	1.21	632	765	0.030
M.F.	I	0.54	857	463	0.021
B.S.	I	0.52	430	224	0.019
R.S.	II	0.55	800	509	0.020
E.J.	II	0.35	682	239	0.011
J.F.	II	0.99	703	696	0.017
E.S.	II	0.75	1046	785	0.018
L.C.	II	0.22	793	175	0.028
A.W.	III	0.65	952	619	0.018
Mean ± (S.D.)		0.60 ± 0.34	735 ± 180	502 ± 238	0.020 ± 0.005

endometrial cancer group was compared to the mean MCR^T (730 ± 53 [S.D.] L./day) of the normal postmenopausal patient group and to a group of 14 normal premenopausal women (675 ± 139 [S.D.] L./day) previously evaluated in our laboratory.[17] There was no significant difference between the mean MCR^T in the endometrial cancer group and the normal premenopausal ($P > 0.4$) and postmenopausal groups ($P > 0.6$).

Production rates of testosterone (PR^T). The mean PR^T (502 ± 238 [S.D.] μg/day) in the endometrial cancer group was compared to the mean PR^T (364 ± 218 [S.D.] μg/day) of the normal postmenopausal patient group and to a group of 14 normal premenopausal women (350 ± 130 [S.D.] μg/day) previously evaluated in our laboratory.[17] There was no significant difference between the mean PR^T in the endometrial cancer group and the normal premenopausal ($P > 0.6$) and postmenopausal groups ($P > 0.1$).

Conversion ratios of testosterone (CR^{TA}_{pp}). The mean CR^{TA}_{pp} (0.02 ± 0.01 [S.D.]) in the endometrial cancer group was compared to the mean CR^{TA}_{pp} (0.04 ± 0.01 [S.D.]) of the normal postmenopausal patient group and to a group of 12 PCOS patients[13] (0.034 ± 0.019 [S.D.]) as previously evaluated in our laboratory. The endometrial cancer group had a statistically lower mean CR^{TA}_{pp} than the post menopausal group ($P < 0.05$).

Comment

During the past decade, there has developed the concept that endometrial cancer is a malignancy associated with an underlying endocrinopathy. The fact that it occurs mostly in women in the postmenopausal age group, in the presence of abnormal and/or unopposed estrogen stimulation, in association with diabetes and obesity, or in women with a history of infertility and anovulation such as in polycystic ovary syndrome, suggests a relationship between the derangement in the endocrine metabolism of these women and the development of the neoplasm.

When the MCR^T, PC^T, and PR^T are compared there is no significant difference noted between the endometrial cancer and the normal postmenopausal patient. Although the mean PR^T of the two groups of patients are not statistically different, there is a suggestive increase in the PR^T in the endometrial cancer patient. The conversion ratio of testosterone to androstenedione, however, is significantly decreased in the endometrial cancer group, as compared to the control group.

The significance of the decreased conversion of testosterone to androstenedione is not known.

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Studies on the cytodynamics of human endometrial regeneration

I. Scanning electron microscopy

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Stereoscopic morphologic studies on regenerating cyclic endometrium indicate that endometrial repair lasts 48 hours, beginning between cycle Days 2 to 3 and ending between cycle Days 4 to 5. During this period, endometrial repair is focused on the neoformation of surface epithelium and is initiated only when and where the zona basalis is denuded from the overlying secretory spongiosa. The surface epithelium is derived from a simultaneous proliferation of dual origin: first from the exposed ends of basal glands and second from the persistent and intact surface lining of cornual and isthmic regions, bordering the denuded areas. The lack of estrogen-dependent morphologic alterations (mitosis and ciliogenesis) in the uterine mucosa during the regenerative period suggests that the initial factors and cellular processes operating in endometrial reconstruction are purely a reflection of tissue repair and are independent of cyclic hormonal stimuli.

MORPHOLOGICALLY, THERE are two reasons for regarding the endometrium as unique and as one of the most dynamic target tissues in the human body. First, the endometrium is subject to hormone-mediated cyclic morphologic modifications which, throughout reproductive life, are programmed to create an optimal environment for the reception and nidation of the fertilized egg. Second, the nongestational endometrium undergoes partial autodestruction and shedding followed by complete reconstruction. In normal menstruating women, both phenomena alternate in a continuous biorhythmic pattern and have a spectacularly rapid turnover rate.

Cyclical endometrial morphologic changes have been extensively investigated by means of light,[1] transmission, and scanning electron microscopy,[2] histo-[3] and ultracytochemistry,[4] Feulgencytophotometry,[5] and historadioautography.[6–8] However, surprisingly little attention has been paid to the study of postmenstrual endometrial repair. Indeed, ever since the first classical description of cyclic changes in human endometrium nearly 70 years ago by Hitschmann and Adler,[9] opinions vary regarding the process of endometrial regeneration and the site of origin of the postmenstrual surface epithelium.

The most accepted view is that the regenerating surface epithelium in human endometrium originates from the residual epithelium of basal gland stumps.[10] Sturgis and Meigs,[11] on the basis of histologic observations, estimated the time renewal of surface epithelium to be 48 hours following the menstrual period. In the opinion of the authors, resurfacing probably occurs by migration of epithelial cells from the exposed ends of the glands. McLennan and Rydell[12] have suggested that the zona basalis remains intact during the menstrual period and that the newly regenerated endometrial surface derives from glandular cells which were involved in the progestational secretory events of the previous cycle. Furthermore, they have suggested that early endometrial regeneration begins as early as the third menstrual day and by the fourth day there are large areas of a new epithelial surface overlying a thin layer of spongiosa. By the fifth day of the cycle, a proliferating surface epithelium is seen throughout the

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uterine mucosa. Concomitantly, the stroma is rapidly restored by mitotically active stromal fibroblasts which are engaged in the production of extracellular collagen.

According to investigators,[12] the ability of secretory endometrium to revert to the proliferative pattern would explain the great speed of endometrial regeneration. However, others[13] believe that the rapidity of epithelial renewal without significant mitotic activity and with nearly constant diploid DNA values[14] in the postmenstrual period does not support the view that the covering surface epithelium regenerates from glandular epithelium which has escaped destruction. In view of these arguments, Baggish and associates [13] favor the concept of transformation of endometrial stromal cells into regenerating surface epithelial cells.

Parallel to morphologic studies on human endometrium, similar investigations have been carried out in experimental animals. Endometrial regeneration was studied in rats,[15] in rabbits following curettage[16] or complete excision of the endometrium,[17] and in the monkey.[18] These studies have shown that the earliest sign of repair, as evidenced by the presence of flattened epithelial cells covering part of denuded surfaces, occurs three hours after curettage.[16] By Day 2[16] to 3[17] the endometrial surface was completely covered by regenerated surface epithelial cells. The resurfacing epithelium was seen to arise chiefly from the persisting surface (in rabbit)[16, 17] or glandular (in monkey)[18] epithelial cells, and mitotic activity became evident only two days following trauma in the rabbit[16] and three days in the monkey.[18] In great contrast to these observations, Singer and co-workers[15] felt that in the rat endometrium regeneration of new surface epithelial cells operates by the transformation of peritoneal cells after their migration through the oviducts.

The presently available data are based solely on histologic observations which, by virtue of their static nature, provide limited and indirect information on the cytodynamics of endometrial renewal. Further and complementary studies with the use of a variety of investigative techniques, such as transmission and scanning electron microscopy as well as histochemistry and radioautography, are therefore needed to obtain additional data on the cellular events which are involved in endometrial repair.

The purpose of this report is to take advantage of the scanning electron microscopy (SEM) by presenting a high-resolution study focusing on the topographic alterations of the human uterine surface epithelium which occur during the process of cyclic endometrial reconstruction.

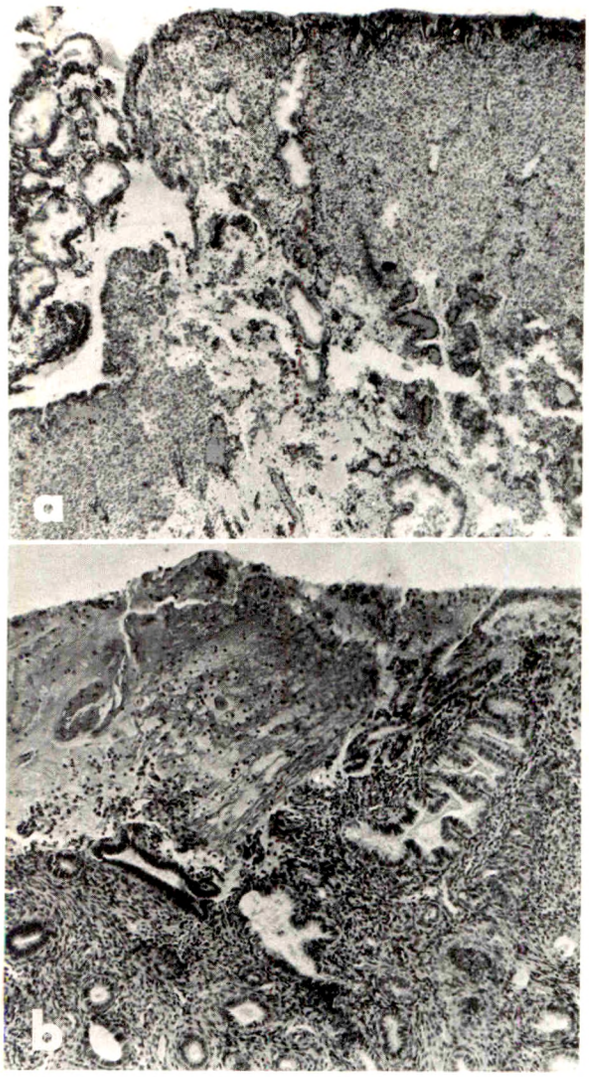


Fig. 1. *a*, Menstrual endometrium, Cycle Day 1. Large fragments of functionalis are dissociated from the underlying endometrium. Hematoxylin and eosin. Original magnification × 35.) *b*, Menstrual endometrium. Cycle Day 2. Portions of denuded zona basalis *(left)* alternate with regions of spongiosa which are still in the process of dissolution. (Hematoxylin and eosin. Original magnification × 65.)

Material and methods

Normal menstruating uteri devoid of gross and microscopic pathology were obtained from a series of 24 patients ranging from 22 to 41 years of age. In order to achieve diagnostic accuracy and reproducibility in dating endometrial specimens, detailed information was secured in each case regarding the dates of at least six previous menstrual periods and basal body temperature (BBT), exact time (as to the hour) of onset of bleeding in progress, cycle pattern in previous years, and time of removal of uteri. The indication for hysterectomy included postconization hysterectomy

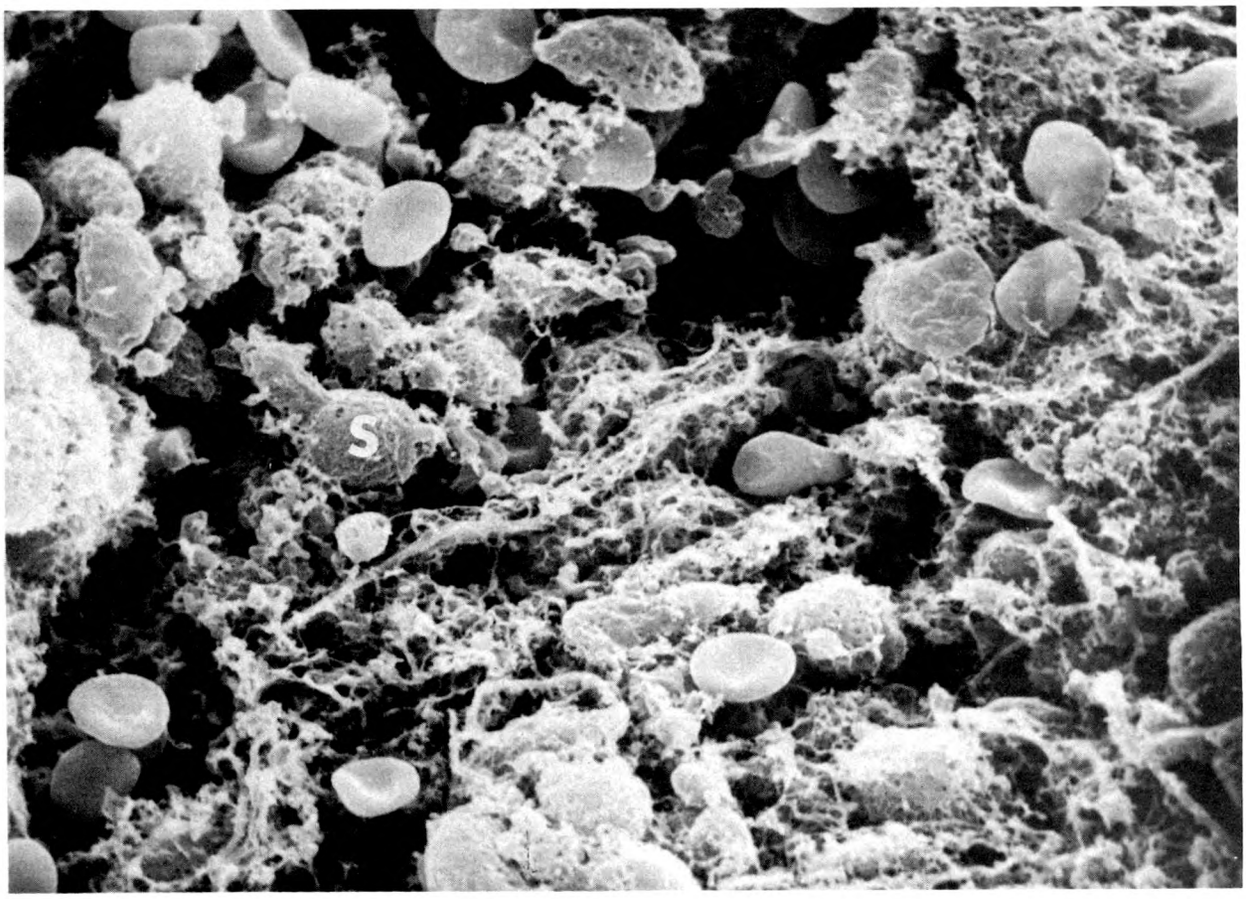


Fig. 2. Menstrual stroma, Cycle Day 2. White and red blood cells are enmeshed in fibrin and reticulum fibers intermingling with degenerated cellular debris, probably of stromal cell *(S)* origin. (Original magnification ×2,000.)

for cervical intraepithelial neoplasia, prolapse of the uterus, and "pelvic congestion syndrome."

Three uteri from each of the first six cycle days starting with the fifteenth day following the rise of BBT, whether or not the first day of menstrual bleeding had occurred, and two uteri from the mid and late proliferative phases of the cycle, respectively, were obtained. All uteri, immediately after their excision, were opened along the lateral borders in order to obtain undistorted anterior and posterior endometrial surfaces. In each cases, under the guidance of a dissecting microscope, the entire uterine cavity was sampled, including the fundal, cornual (peritubal), and isthmic portions. Blocks of tissue measuring 2.0 by 1.0 by 0.5 cm. including the basalis were submitted for scanning and transmission electron microscopy[21] and light microscopy, and were processed and examined as previously described.[2] In an effort to achieve maximum confidence in identification of tissue examined with SEM, scanned specimens were subsequently rehydrated and verified histologically. For rehydration, samples were placed in 100, 95, 80, 70, and 50 per cent alcohol, each step lasting three hours, and then in distilled water. Following Bouin's fixation, they were placed and processed in an autotechnicon in the usual fashion. The results of rehydrating critical point dried specimens were adequate in evaluating their histologic appearance.

Results

Because morphologic alterations during the menstrual phenomenon varied among specimens obtained on the same cycle day (at the same time) as well as within the same uterine cavity, accurate correlation between morphology and time of removal was impossible. For this reason, the sequence of events will be described and evaluated according to the day (24 hours period) during which the specimens were recovered.

Uteri removed on the first and second menstrual days contained histologic (Fig. 1, *a* and *b*) and scanning



Fig. 3. Isthmic and uterine corpus junction, Cycle Day 2. Electron micrograph illustrating the well-preserved surface epithelium of the isthmus *(LUS)* bordering the denuded basal layer in which the gland mouths are greatly dilated (arrow). (Original magnification ×180.) Inset, Light micrograph of the junction of intact LUS *(left)* and denuded zona basalis. (Hematoxylin and eosin. Original magnification ×60.)

electron microscopic (Fig. 2) evidence of surface epithelial, stromal and glandular fragmentation, and disintegration with extravasation of red blood cells and inflammatory exudate. These alterations involved both the compacta and spongiosa and occurred chiefly in the fundal and corporeal regions of the uterine cavity. As a result, large fragments of functionalis were seen to be dissociated from the underlying tissue (Fig. 1, *a*). Although the degree of endometrial disruption displayed no topographic uniformity in uteri obtained on the same cycle day, tissue destruction and loss were, in general, more conspicuous on the second than on the first menstrual day. Indeed, two of three specimens obtained on Day 2 of the cycle exhibited considerable tissue loss resulting in large zones of denuded basal layer (Fig. 2, *b*). In these regions the hemorrhagic stroma had numerous, wide, open gland stumps, a few of them showing curved edges with occasional short epithelial extensions onto the surrounding tissue. These changes are regarded as the earliest sign of surface epithelial reconstruction.[19] Examination of the uterine mucosa of the isthmic and cornual segments revealed foci of stromal hemorrhage and inflammatory exudate but the endometrial architecture, including the surface epithelium, remained intact (Figs. 3 and 4). The latter, with the SEM, was composed of well-preserved ciliated and dome-shaped nonciliated cells with hairlike microvilli and occurred in a ratio of approximately 1:30 (Fig. 4, inset).

On the third menstrual day, most of the basal zone appeared denuded, containing only a few foci of the residual spongy layer. It had an irregular, ragged surface because of the extent of endometrial shedding. While many basal gland crypts were oriented parallel to the long axis of the uterine cavity, their body and neck were arranged obliquely or perpendicular to the

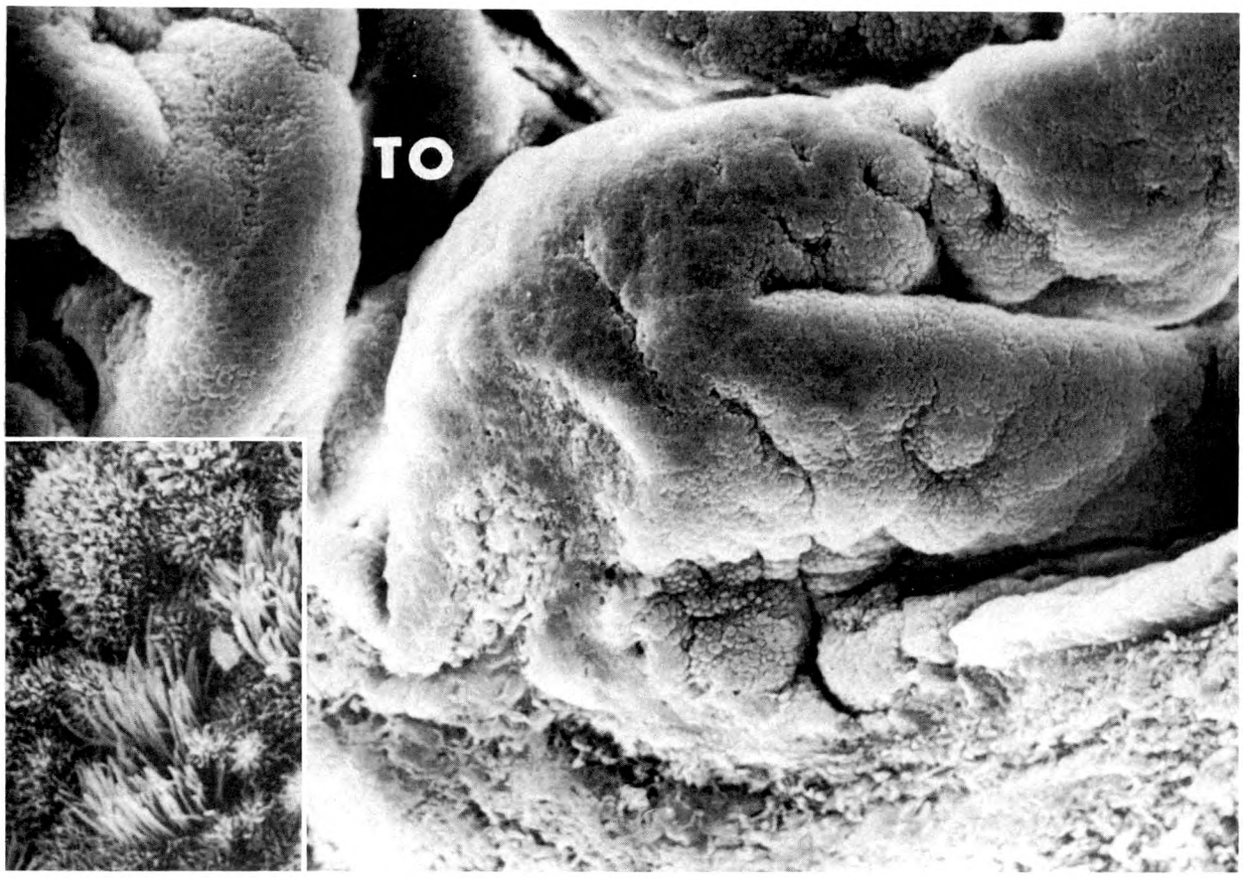


Fig. 4. Fundus-cornual junction, Cycle Day 2. The mucosa near the tubal ostium *(TO)* appears well preserved with an undulating surface and irregular edges bordering the zona basalis of the fundus. (Original magnification ×230.) Inset, Higher magnification of intact ciliated and dome-shaped microvillus cells lining the peritubal region. (Original magnification ×3,000.)

surface into which opened haphazardly spaced gland mouths (Fig. 5, *A*). In contrast to the previous day of the menstrual period, specimens of the third menstrual day had most of their gland stumps with rolled edges, from which varying sized and randomly oriented epithelial masses extended onto the adjacent stroma (Fig. 6). In several areas, adjacent gland mouths were interconnected by surface epithelial bridges. Light microscopic verification of rehydrated SEM specimens revealed the epithelial surface extensions to be continuous with the neck of basal glands, providing histologic confirmation of their site of origin (Fig. 6, inset). The topographic architecture of developing surface epithelium exhibited a certain degree of cellular pleomorphism (Fig. 7, inset). In addition, cellular cohesion and organization were impaired, as compared to the fully reconstructed surface lining of the mid and late proliferative phase of the cycle. The surface plasma membrane of migrating epithelial cells was often wrinkled or flattened and the microvillous processes were short and sparse. Ciliary shafts were not observed in these cells. At the light microscopic level, the epithelium was cuboidal to low columnar near the glandular ostia and decreased in height near its distal end. In this region the epithelial cells were flat to spindle shaped with dark cytoplasmic matrix and the nuclei were enlarged and hyperchromatic. There was no histologic continuity between the resurfacing epithelial and underlying stromal cells, as evidenced by the presence of a well-defined basal membrane separating the two-cell populations. During Day 3 of the cycle, the periphery of the endometrial surface of the isthmic and cornual segments showed irregular tongues of surface epithelium extending onto the adjacent and denuded zona basalis of the body and fundus, respectively. Both the surface ultrastructural and light microscopic characteristics of this advancing epithelium were similar to those found in its counterpart of basal gland neck origin.

Uteri possessed comparatively better developed

surface epithelial extensions on Cycle Day 4 than on the previous cycle day (Fig. 7). These, by centripetal expansions and ramifications were seen to merge into and eventually fuse with their adjacent counterparts. Simultaneously, tongues of surface epithelium of isthmic and cornual origin established anastomoses with the peripheral portions of the surface lining originating in the gland stumps of the zona basalis. As a result of rapid surface re-epithelialization, most denuded areas were obliterated on Day 4 of the cycle (two of three specimens had fully repaired surface covering), coinciding with the decrease and eventual cessation of endometrial shedding and menstrual flow. Histologically, the subepithelial stroma was loose, edematous with scattered spindle-shaped fibroblasts devoid of mitotic figures (Fig. 5, *B*).

Specimens received on Cycle Days 5 and 6 had an entirely reconstructed surface epithelium, covering an irregular and often hemorrhagic basal layer. The lining epithelium contained randomly distributed glandular ostia around which ciliated cells were concentrated in a crownlike fashion (Fig. 8). The closely apposed nonciliated cells had a penta- to hexa-gonal contour, and the distended surface plasma membrane was covered by short and stubby microvilli (Fig. 8, inset). Histologically, the surface epithelium varied from cuboidal to tall columnar, and the nucleocytoplasmic ratio was in favor of the cytoplasm. Cells with cilia and mitoses were scattered throughout the surface epithelial membrane, with greater concentration in the periglandular region and the upper half of the glandular epithelium. Serial histologic sections of endometria obtained on Cycle Days 5 and 6 demonstrated short, straight basal glands with narrow lumina, connecting the surface and the basal layer.

Uteri obtained on Cycle Days 8 and 10 (mid-proliferative phase of the cycle) were covered by an undulating surface membrane in which numerous ciliated cells, either single or in clusters, were found not only in a periostial location but also on the interglandular surface areas (Fig. 9). The bulging nonciliated cells were covered by abundant, 0.5 μ long microvillous projections with occasional microvillous promontories (Fig. 9, inset). Several glands were seen to divide at the junction of the basalis-spongiosa layers, and the stromal cells displayed numerous mitotic divisions. Because of accelerated epithelial and stromal cell growth, the number and length of endometrial glands and their supporting stroma rapidly increased, leading to the over-all expansion of the uterine mucosa.

The endometrial surface of the late proliferative phase (twelfth to fourteenth day of the cycle) contained a further increase in the number of ciliated cells which were arranged in large clusters alternating with microvillous nonciliated cells in a ratio of about 1 to 30.

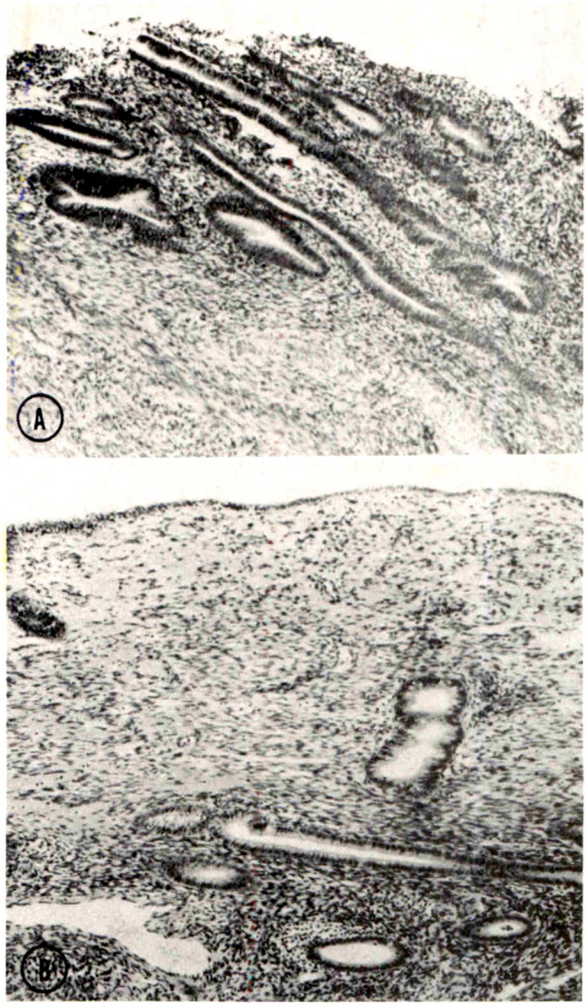


Fig. 5. *A*, Regenerative endometrium, Cycle Day 3. The zona basalis is completely denuded from the overlying spongiosa and contains obliquely oriented glands surrounded by a compact stroma. (Hematoxylin and eosin. Original magnification ×85.) *B*, Regenerative endometrium, Cycle Day 4. Newly formed surface epithelium covers the thin uterine mucosa in which the stroma is loose and glands are sparse. (Hematoxylin and eosin. Original magnification ×73.)

Comment

The present SEM study on regenerating human endometrium concurs with earlier histologic investigations[11, 12] showing that the total generation time for postmenstrual surface re-epithelialization is about 48 hours. The regenerative period begins between Cycle Days 2 and 3 and ends between Cycle Days 4 and 5. This time interval coincides with the maximum area of denuded zona basalis.

Surface epithelial repair occurs by a simultaneous

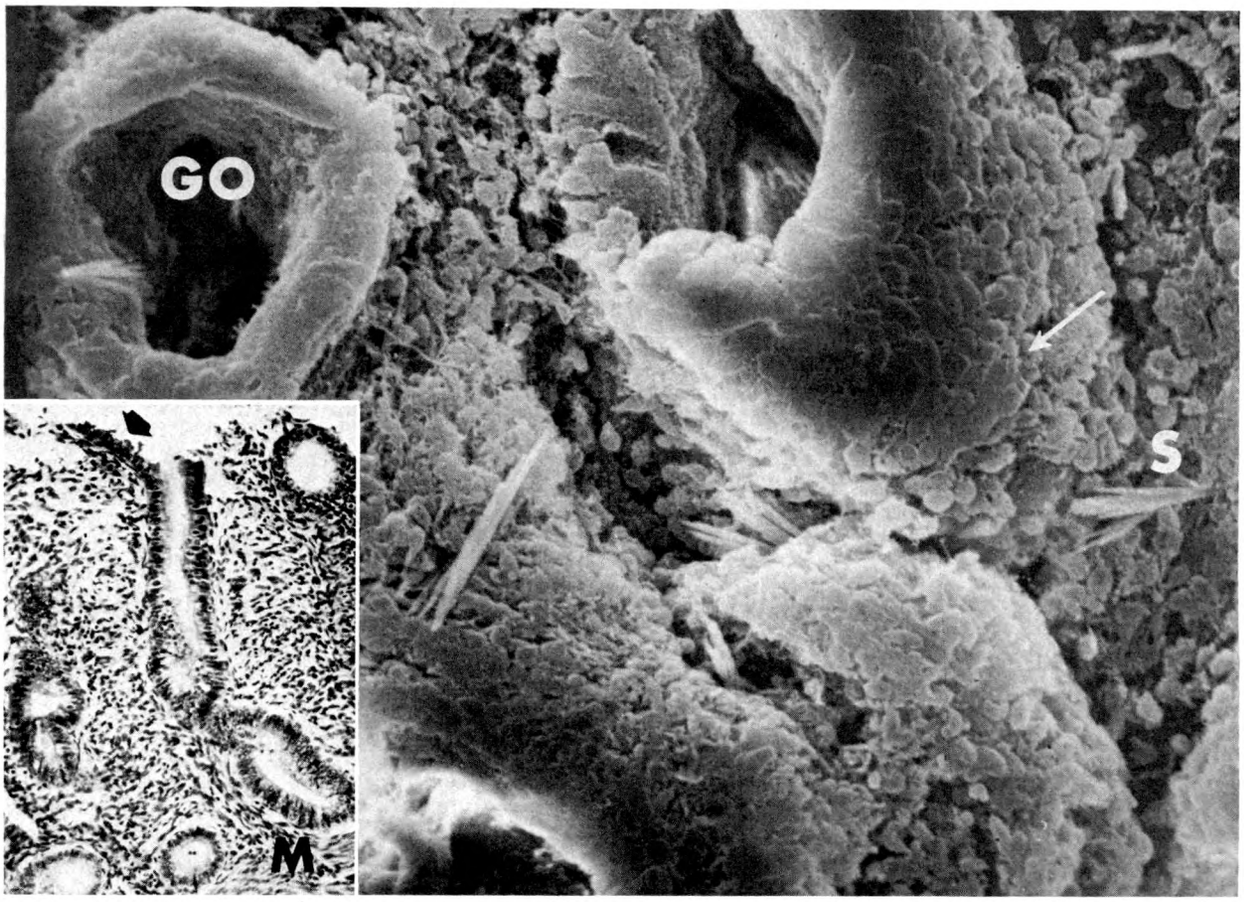


Fig. 6. Regenerative endometrium, Cycle Day 3. The gland stumps *(GO)* of the zona basalis have prominent openings with rolled edges and epithelial extensions *(arrow)* on the adjacent stroma *(S)*. Original magnification ×380. Inset, Histologic appearance of rehydrated SEM specimen shown in the figure. The resurfacing epithelial extension (arrow) is continuous with the lining epithelium of the basal gland. *M*, Myometrium. (Hematoxylin and eosin. Original magnification ×210.)

and progressive epithelial outgrowth from the mouths of basal glands and by ingrowth from the surface epithelium adjacent to the denuded areas, namely, the isthmic and cornual regions. Both the basal glands and surface epithelium of peripheral uterine regions remain intact during the entire menstrual period. On Day 4 of the cycle, the rapid development of interanastomoses between converging epithelial proliferations leads to complete or near-complete restoration of the new surface epithelium. By the fifth day of the cycle, the endometrial surface is fully re-epithelialized.[19, 20] These observations indicate that while the first half of menstruation is dominated by tissue destruction and loss, reconstruction prevails in the second half. The latter period, therefore, is recognized as the regenerative phase of the menstrual cycle.

Furthermore, the results indicate that the regenerating surface epithelium has a dual rather than a single anatomic[10, 13, 18] origin — first, and in agreement with most previous light microscopic studies,[10, 11, 18] in the exposed stumps of the basal glands and second, in the surface epithelium of isthmic and cornual regions. With regard to this surface epithelium, a somewhat similar situation was reported in the rabbit's endometrium,[16, 17] in which the newly repaired surface lining derived from the intact surface epithelium bordering the artificially denuded area. In the woman, the proliferative activity and considerable number of epithelial extensions of basal gland origin are believed to represent the dominant mechanism of endometrial surface renewal. The basal glands are the main source of the new surface epithelium.

Certain investigators[12] have expressed the view that endometrial healing develops by rapid metamorphosis of the residual secretory endometrium to proliferative tissue. According to these authors, such a process would also explain the normal length cycle with prolonged or delayed period of menstrual shedding.

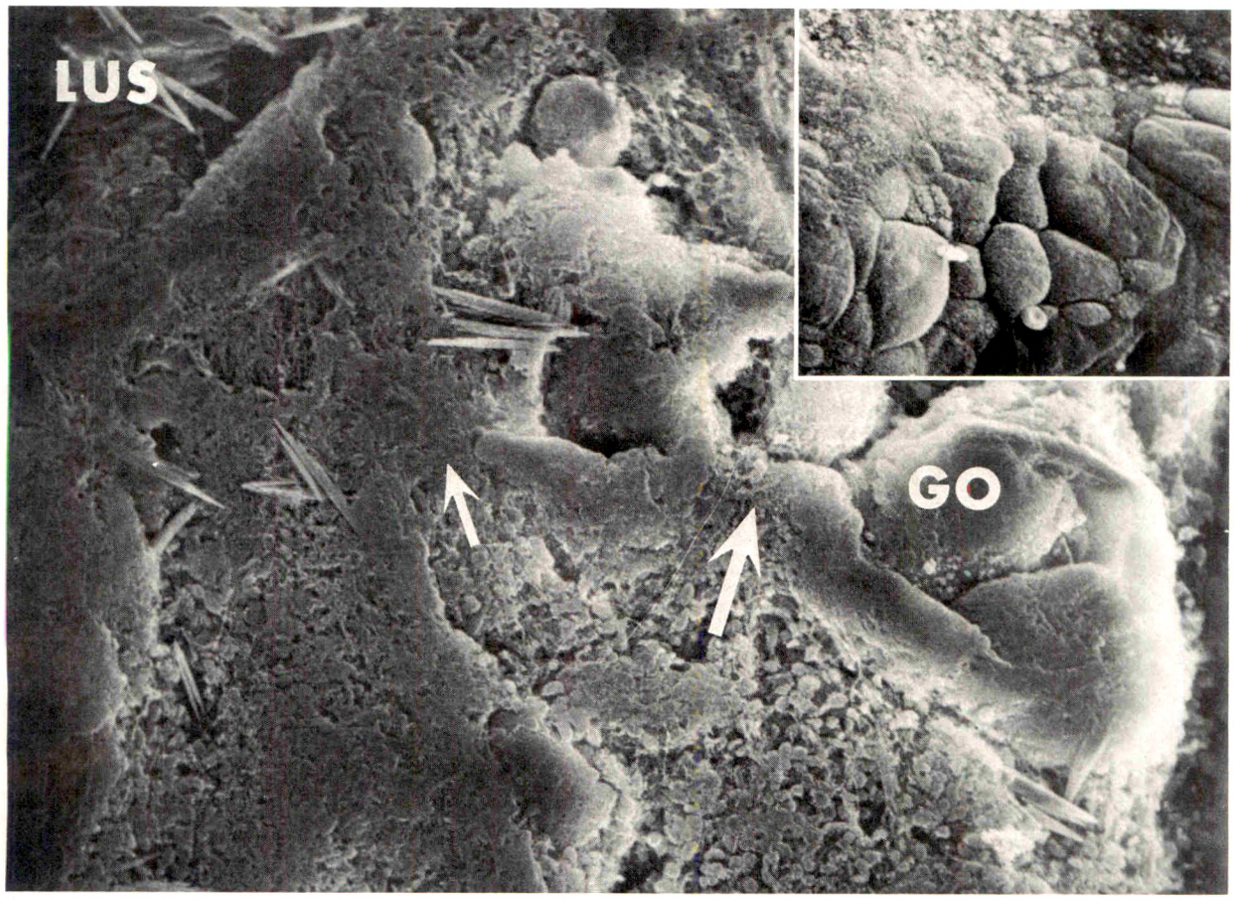


Fig. 7. Regenerative endometrium, Cycle Day 4. Anastomoses between surface epithelial extensions of basal gland and isthmus origin (short arrow) as well as between adjacent gland openings *(GO)* are established (large arrow). Because of inadequate washing of specimens following fixation with phosphate-buffered glutaraldehyde, needlelike structures representing phosphate-crystals remain on the surface. (Original magnification ×230.) Inset, The developing surface epithelium is made of cells of various sizes and shapes with generally flattened surface plasma membrane. (Original magnification ×900.)

The residual progestational endometrium would "catch up" with the proliferative endometrium later in the cycle. Our studies, however, suggest that surface epithelial restoration is initiated only when and where gland stumps of the basal layer are denuded from the overlying spongiosa. This phenomenon, together with the segmental rather than the diffuse nature of menstrual dissolution, would account for the focal distribution of resurfacing epithelium and for the alternating pattern of regenerating and disintegrating regions. Thus, in agreement with McMinn,[18] " . . . regeneration begins before menstruation ceases" and terminates by the fifth day of the menstrual cycle. Assuming that loss of secretory spongiosa is a prerequisite for stimulation of endometrial repair, prolonged menstrual bleeding may be related, at least in part, to delayed separation of the functionalis from the basalis. Similarly, the superficial loss of uterine mucosa, not extending to the basalis, may partly be responsible for prolonged anovulatory bleeding. In both situations denudation of the basal layer would be impaired, precluding rapid surface reepithelialization and thus promoting abnormal uterine bleeding. Consequently, once the basal zone is freed either endo- or exogenously (curettings) of the overlying tissue, epithelial regeneration will resume and bleeding will cease. Admittedly, a good number of detailed investigations of uteri removed from patients with history of various abnormal bleeding is needed to substantiate our hypothesis.

Contrary to earlier suggestions,[9, 12, 13] we were unable to demonstrate morphologic features (transformation of stromal cells into surface epithelial cells,[13] collagen synthesis, or mitosis in stromal cells[12] that gave

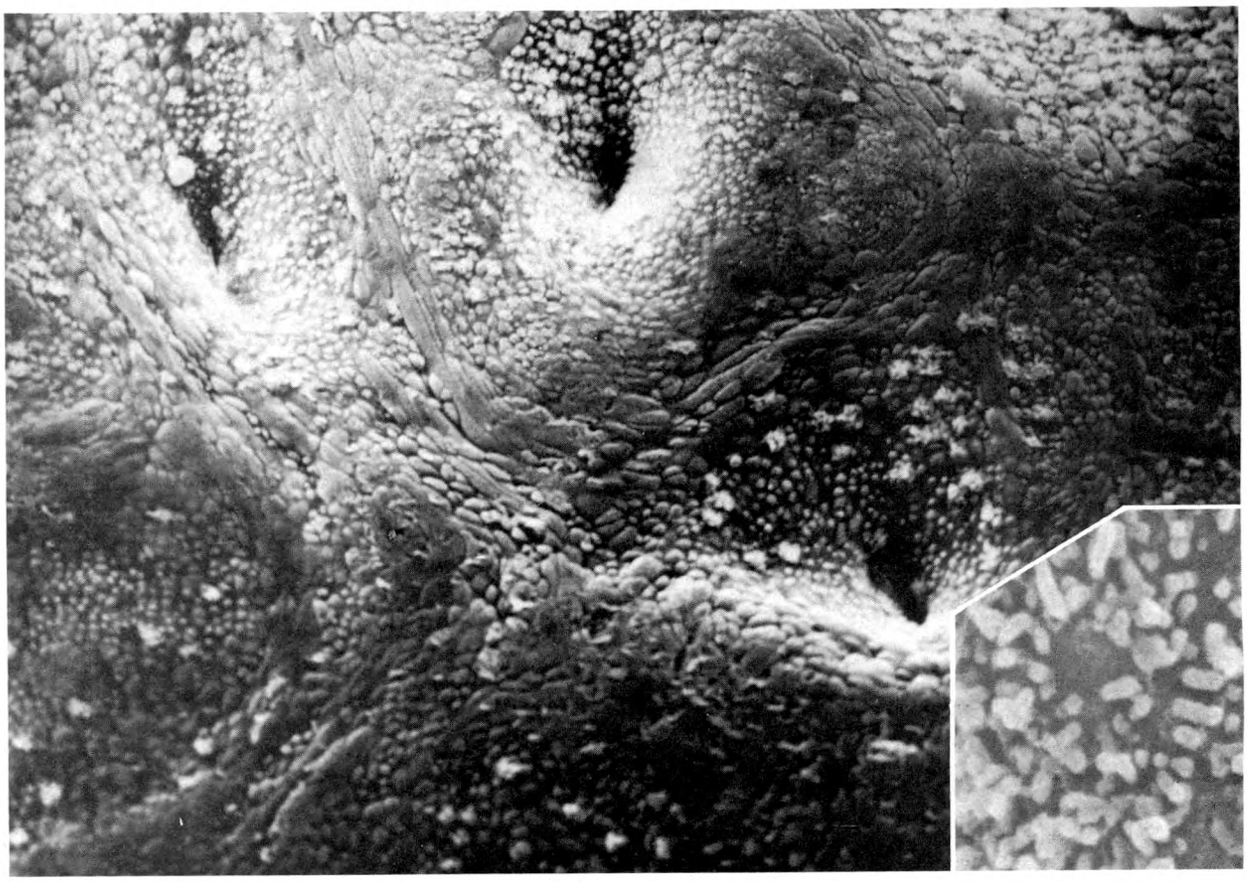


Fig. 8. Early proliferative endometrium, Cycle Day 5. The surface is fully reconstructed and contains several glandular openings, typically surrounded by ciliated cells. The interglandular surface is composed of microvillus cells of various sizes having a cobblestone appearance. (Original magnification ×360.) Inset, High magnification of short, stubby, and sparse microvilli. (Original magnification ×11,500.)

evidence of significant participation of stromal cells assisting in the formation of resurfacing epithelium or restoration of the uterine stroma during the regenerative period. In fact, our SEM and preliminary transmission electron microscopy and histochemical studies[21] reveal that the predominant proliferative activity during endometrial regeneration is confined to generation of the surface epithelium. The stromal cells remain inactive until surface re-epithelialization is completed by Cycle Day 5. Since estrogen-dependent changes, such as mitosis and ciliogenesis, are not seen before the surface epithelium is restored, one can assume that endometrial surface repair is initiated by, and is in response to, tissue loss rather than under the influence of ovarian hormones. This contention seems to be supported by studies on endometrial regeneration in hormonally intact experimental animals[16, 17] in which proliferation of a new surface epithelium is reported to begin as early as three hours after artificially induced endometrial injury. Moreover, the lack[1, 13] of epithelial mitoses during the period of regeneration suggests that the developing epithelium proliferates by either amitotic division or endoreduplication or that the S phase of mitosis is so accelerated as to escape morphologic detection. The newly formed epithelial cells may possibly migrate by ameboid motion over the denuded stroma.

At this point, however, several questions remain to be clarified:

1. What are the dynamics of the subcellular sequence of events in both the epithelial and stromal cells during the process of repair in cyclically shedding endometrium?

2. What is the precise mechanism of endometrial proliferation during endometrial reconstruction in the absence of significant mitotic activity?

3. What is the rate and distribution of DNA synthesis and renewal time of surface epithelial cells as well as

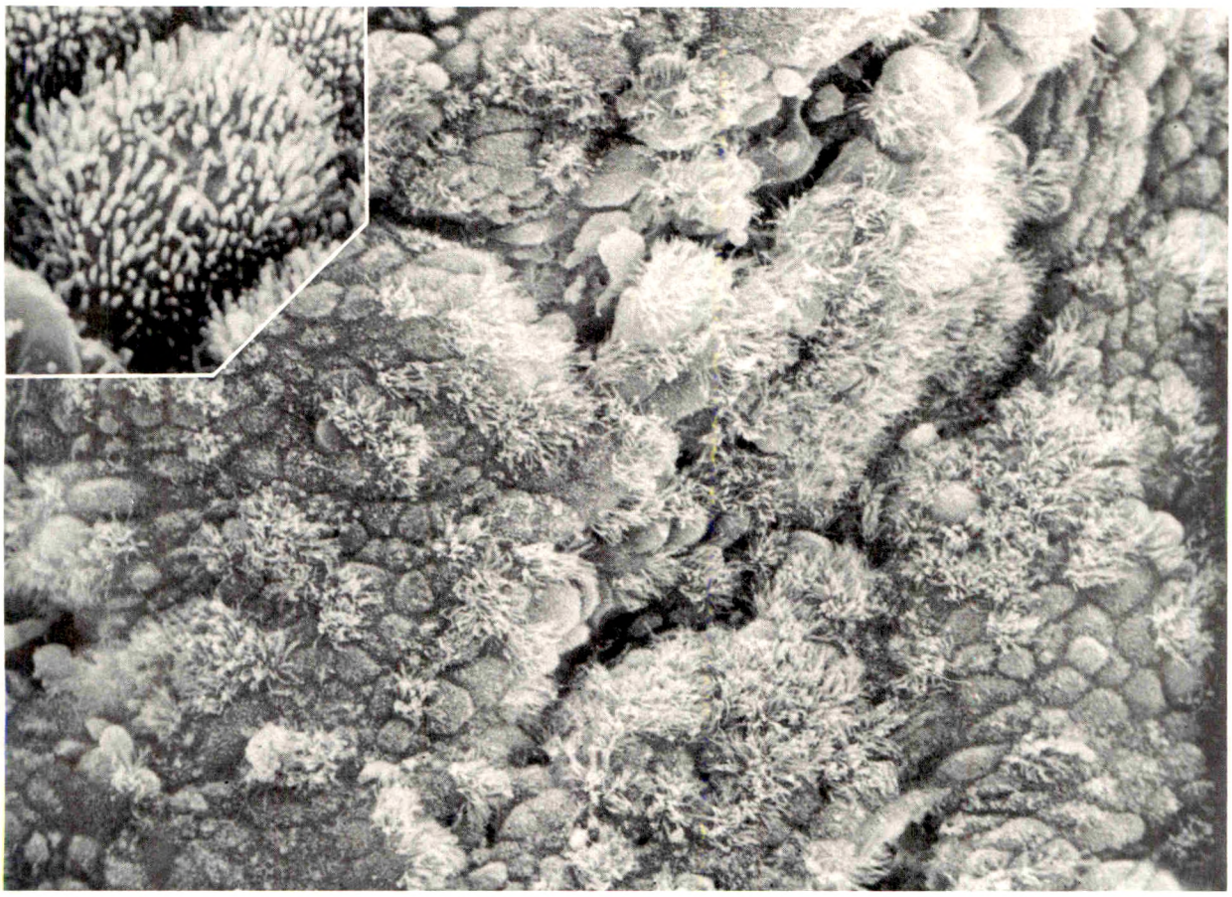


Fig. 9. Mid proliferative endometrium, Cycle Day 10. By Cycle Days 9 to 10, ciliated cells become visible on the interglandular surface plateaus, arranged in clusters or as single units. (Original magnification ×850.) Inset, Detailed view of dome-shaped cells covered by numerous, well-developed microvillus processes. (Original magnification ×7,800.)

glandular and stromal cells during the regenerative period?

Experiments with the use of transmission electron microscopy[21] and historadioautography are underway to elucidate these questions, to test certain details of our hypotheses, and to substantiate the presently available morphologic observations concerning the regenerative process of cyclic endometrium.

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Pituitary responses to LRH in the postpartum periods

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Pituitary responses to luteinizing-hormone–releasing-hormone (LRH) in the postpartum periods were studied. Following a subcutaneous injection of 100 μg of synthetic LRH to postpartum subjects, no statistically significant changes in the levels of LH and FSH could be demonstrated in five subjects on postpartum day 1 or 3 and in three subjects on postpartum day 8. A normal elevation of LH and FSH following LRH was demonstrated in one subject 36 days post partum. The findings are in agreement with previous studies demonstrating a persistence of pituitary suppression during the early postpartum period. No correlation could be drawn between the pituitary responses to LRH and the plasma levels of estradiol-17β and progesterone.

WITH PREGNANCY, suppression of the hypothalmic-pituitary axis occurs as a result of the abundant steroid hormones produced by the fetoplacental unit. Following parturition the high levels of estrogen and progesterone drop precipitously.[1] The levels of LH-HCG similarly decreases,[2] as do prolactin levels in non-nursing females.[3] This alteration in hormonal milieu is probably responsible for the delayed resumption of the ovulatory cycle post partum.[4] Synthetic LRH has repeatedly been shown to stimulate the pituitary to release LH and FSH in nonpregnant females.[5, 6] as well as during pregnancy.[7] Recently, limited studies concerning the pituitary response to LRH postpartum have been reported.[8, 9] The purpose of this study is to further investigate the changes in pituitary responsiveness to LRH during the postpartum period and to correlate with plasma estradiol and progesterone levels.

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Materials and methods

The five subjects of this study were all patients at the Chicago Lying-in Hospital ranging in age from 17 to 28 years. All had a previously normal menstrual history and no endocrinologic abnormalities were noted in history or physical examination. All subjects had a spontaneous vaginal delivery at term. Medications at the time of the study included the usual analgesics for postpartum patients, one subject (D.B.) received antibiotics. Consents were obtained from all participants prior to delivery. No lactation-suppressive drugs were given and none of the patients breast fed.

An indwelling catheter was placed in the antecubital vein and kept open with isotonic saline infusion. After baseline blood samples were taken at −30 minutes and zero time, a single dose of LRH 100 μg (Ayerst AY-24031) was injected subcutaneously. Blood samples were then collected at 30 minute intervals for 2 hours and at hourly intervals thereafter.

Prior to LRH injection, additional blood samples were obtained for the measurement of estradiol-17β and progesterone. Blood samples were collected at 24 hours (postpartum day 1) and at 72 hours (postpartum day 3) after delivery, and on postpartum days 8 and 36 sampling started between 8 and 9 A.M. All five subjects participated on days 1 and 3, three subjects participated on day 8, and one subject participated on day 36. Plasma and serum were subsequently separated and

Table I. Changes in FSH levels* before and after LRH injection on postpartum day 1, 3, and 8

Postpartum Day	*Time† (min.)*	*Subjects*				
		R. G.	*V. H.*	*D. B.*	*N. R.*	*L. B.*
1	−30	56	ND‡	74	30	ND
	0	80	ND	44	ND	ND
	+30	92	ND	58	56	ND
	+60	152	ND	58	24	ND
	+90	66	ND	44	ND	20
	+120	100	ND	44	30	32
	+180	138	10	68	52	56
	+240	92	24	30	ND	32
	E_2 (pg./ml.)§	322	205	252	110	67
	P (ng./ml.)‖	4.4	2.7	2.0	3.2	1.8
3	−30	53	30	24	34	50
	0	40	20	ND	40	37
	+30	50	30	ND	80	40
	+60	92	ND	ND	40	70
	+90	40	ND	ND	52	56
	+120	92	ND	ND	26	52
	+180	140	ND	ND	28	88
	+240	132	ND	ND		30
	E_2 (pg./ml.)	61	158	111	30.7	38
	P (ng./ml.)	0.5	1.0	0.4	1.02	0.4
8	−30	42	66	32		
	0	26	44	20		
	+30	68	88	22		
	+60	32	60	36		
	+90	100	76	20		
	+120	80	134	22		
	+240	108	80	20		
	E_2 (pg./ml.)	—	210	63		
	P (ng./ml.)	—	1.7	0.1		

*FSH in terms of ng./ml. (LER-907).
†Time, in minutes, before (−) and after (+) LRH injection.
‡ND indicates "nondetectable".
§Estradiol-17β.
‖Progesterone.

kept frozen at −18° C. until assayed. Each individual's samples for a given day were analyzed in the same assay. FSH and LH-HCG (HCG cross-reacts in the LH assay) were measured by a double antibody radioimmunoassay as described by Odell and associates[10] and expressed as nanograms per milliliter using LER 907 distributed by NIAMD, NIH, as the standard reference. In our gonadotropin assay system the intra- and interassay coefficients of variation are 5 and 20 per cent respectively. The limit of sensitivity is 10 nanograms per milliliter for both LH and FSH. Plasma levels of estradiol-17β and progesterone were determined as previously reported.[11]

Results

Baseline levels of FSH and LH. The results of FSH and LH concentration are shown in Tables I and II. In all subjects the levels of FSH on postpartum days 1, 3, and 8 are significantly below those of normal follicular phase (80 to 410 ng. per milliliter). Of the five subjects, three on day 1 and two on day 3 had no detectable levels of FSH in multiple samples. On day 8 all had detectable FSH values. The levels of FSH in one subject (L.B.) on postpartum day 36 was in the normal follicular phase range.

LH-HCG concentrations ranged widely for day 1 (1,820 to 2,330 ng. per milliliter) and for day 3 (860 to 2,380 ng. per milliliter), and declined with time. The level of subject L. B. at 36 days was in the follicular phase range (30 to 210 ng. per milliliter).

Effects of LRH. Table I lists the values of FSH following injection of LRH. Increases of 200 per cent or greater above the mean baseline were noted on day 1 and 3 in two of five subjects (N. R. and R. G.) at 30 and 60 minutes, respectively. Secondary elevations in FSH levels, all at 180 minutes after LRH injection, exist in three subjects (R. G., L. B., and N. R.,) on day 1, and in two of these subjects (R. G. and L. B.) on day 3. In

Table II. Changes in LH levels* before and after LRH injection on postpartum day 1, 3, and 8

Postpartum day	Time† (min.)	Subjects				
		R. G.	V. H.	D. B.	N. R.	L. B.
1	−30	2,080	2,540	2.640	2,640	2,880
	0	2,060	2,500	2,500	2,700	2,740
	+30	1,990	2,340	2,620	2,620	2,820
	+60	1,960	2,460	2,540	2,640	2,780
	+90	2,000	2,460	2,640	2,440	2,780
	+120	2,000	2,280	2,620	2,540	2,800
	+180	1,980	2,300	2,580	2,540	2,700
	+240	1,920		2,460	2,540	2,740
3	−30	950	1,354	2,380	1,146	1,880
	0	860	1,250	2,360	1,120	1,800
	+30	850	1,320	2,220	1,090	1,920
	+60	830	1,220	2,300	1,116	1,920
	+90	830	1,210	2,320	1,130	1,980
	+120	920	1,186	2,300	1,110	1,890
	+180	820	1,160	2,240	1,080	1,920
	+240	804	1,360	2,220		1,940
8	−30	12.6	560	350		
	0	15.4	586	344		
	+30	15.0	590	294		
	+60	14.4	500	340		
	+90	26.0	600	340		
	+120	17.0	526	314		
	+240	14.6	540	280		

*LH in terms of ng./ml. (LER-907).
†Time, in minutes, before (−) and after (+) LRH injection.

subject R. G. on day 8 a prolonged rise of FSH occurred some time after 60 minutes following LRH injection. With the use of a one-tailed Student t test, as a group, no statistical difference is demonstrable for the various peaks.

In contrast, no spikes in the levels of LH-HCG occurred following LRH administration on day 1, 3, or 8, as demonstrated in Table II. A wide range of LH concentrations was observed in the study subjects.

Fig. 1 shows the results in subject L. B. at 36 days post partum. A sustained elevation of FSH occurred at 30 minutes, reaching its maximum value at 120 minutes. The level of LH increased at 30 minutes after injection, peaking at 60 minutes (greater than sevenfold increase) and declined to baseline levels at 240 minutes.

Estradiol-17β and progesterone. Estradiol and progesterone values are listed along with FSH values in Table I. The sample for R. G. on day 8 was inadvertantly destroyed prior to analysis. A general trend of decreasing values of E_2 and P was demonstrated with increasing days post partum. The levels of E_2 and P returned to the range of follicular phase (21 to 120 pg. per milliliter and < 1.5 ng. per milliliter, respectively) by the third postpartum day except for subject V. H., in whom elevated E_2 and P

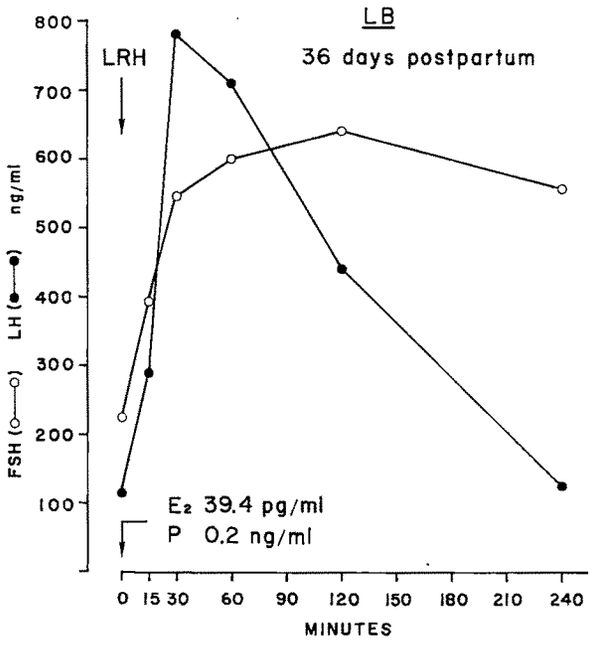


Fig. 1. Levels of FSH and LH after LRH 100 μg subcutaneously in a patient 36 days post partum.

values persisted up to postpartum day 8. No correlation could be drawn between the pituitary responses to LRH and the plasma levels of estradiol-17β or progesterone.

Comment

Zarate and associates[7] previously reported that FSH values are detectable throughout pregnancy. In a recent report by Canales and associates,[9] FSH values in all 11 patients studied were nondetectable 2 days post partum, before and after a 50 μg intravenous bolus of LRH (Hoechst). We have found detectable FSH levels on days 1 and 3 in all subjects, though some individual samples showed nondetectable FSH levels on day 1 and in two individuals on day 3 post partum. The use of lactating subjects by Canales and our choice of nonlactating subjects may account for some of the variance between the two reports. In agreement with Canales, however, is the notably low level of FSH postpartum.

Values obtained from the LH assay due to cross-reactivity with HCG were markedly elevated post partum. Despite the progressive disappearance of HCG, significant interference was still present at 8 days post partum, which is in agreement with other investigators.[12] The values of LH for our one subject at 36 days post partum is in the range of normal follicular phase, most likely representing pituitary LH rather than residual HCG.

Zarate and associates[7] intravenously injected 50 μg of LRH at various stages of pregnancy and reported FSH peaks occurring in nine of 10 subjects within the first 64 minutes. They concluded that the pituitary remained responsive during pregnancy to hypothalamic releasing hormones, finding 10 of 13 subjects who did respond to LRH injections. Canales and associates,[9] again using 50 μg of LRH intravenously, found no effect on LH or FSH release at 2 days post partum. At 2 weeks post partum the pituitary was responsive to LRH, showing significant changes in FSH and LH values, LH peaking at 30 minutes post injection and FSH reaching its maximum between 30 and 60 minutes and declining thereafter. The present study and that of LeMaire and associates[8] used 100 μg LRH (Ayerst) subcutaneously. Arimura and his associates[6] had previously studied the course of LH and FSH in normal males after subcutaneous injection of 100 μg of LRH. LH values were seen to peak between 30 and 45 minutes with a secondary spike appearing at 180 minutes. LeMaire and associates,[8] observing only LH response, found no change at 2 weeks postpartum but a significant response at 6 weeks. In the present study two of five subjects on day 1 and 3 post partum demonstrated elevations of FSH values at or before 60 minutes following a LRH challenge, with secondary increases being noted at 180 minutes in both subjects on day 1 and in one of the two subjects on day 3. These time periods of peaks are in agreement with the findings of Arimura and associates.[6] At 8 days post partum the minimal elevations noted appear much later following LRH injection. As described previously, none of these elevations was of statistical significance. The response of one subject at 36 days post partum appears to correlate well with the observation by LeMaire and associates[8] at 6 weeks post partum.

It is well known that the menstrual period is generally delayed following conception, the delay being longer for those mothers who are lactating. The cause for the delay remains unsettled. Zarate and associates[13] injected human menopausal gonadotropins into postpartum volunteers and found no elevation of estrogen or progesterone, concluding that the ovaries were the primary refractory organ. Crystle and colleagues[14] found that estrogen in low doses would produce elevation in LH and FSH concentrations post partum in comparison to controls and patients receiving high-dose estrogen supplementation. They hypothesized that the ovaries were the primary refractory organ and that pituitary stimulation post partum was dependent on low-dose estrogen priming. During the normal menstrual cycle estrogen and progesterone appear to have a priming effect on the pituitary following LRH injections during the midcycle and the luteal phase.[5, 14–16] Yen and associates[5] proposed that estrogen and progesterone have an additive effect in enhancing LH-FSH release and synthesis. In contrast, Thompson and associates[17] and Kastin and associates[18] found that synthetic estrogen apparently depressed pituitary secretion of LH and FSH following LRH challenge, though normal qualitative responses were obtained. Thompson and associates[17] also reported similar results with progesterone. The possibility of pituitary sensitivity dependent on various ratios of hormones or concentrations was raised to explain the variance between estrogen and progesterone supplementation and the normal menstrual cycle.

In a sense our results may resemble those obtained with estrogen and progesterone supplementation, qualitatively normal but of decreased magnitude. However, the small number of subjects demonstrating FSH surges, the high interassay coefficient of variation, and the substantially decreased size of the FSH surge, leave doubt concerning any responsiveness of the pituitary in the early postpartum period.

Last, we attempted in a limited number of subjects to show some relationship between responders and plasma levels of estradiol-17β or progesterone. Despite the levels of E_2 and P returning to the follicular phase levels in the majority of the subjects studied during the early postpartum period, no pituitary responses to LRH administration similar to nonpregnant women were demonstrated. It suggests an altered sensitivity of the postpartum pituitary due to a prolonged suppression. However, the possible effects of persistently high concentrations of sex-steroid binding globulin in the postpartum period on the available endogenous E_2 should also be considered. It raises the need for further study of the causes of variance at the time of resumption of normal pituitary responsiveness in a larger population with more frequent sampling of hormonal levels including prolactin.

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C. S. Mott Center Colloquium in Andrology: Techniques of Human Andrology

The C. S. Mott Center for Human Growth and Development is holding a colloquium on Techniques of Human Andrology, May 6-7, 1976, at Wayne State University School of Medicine, Detroit, Michigan. Research papers on techniques will be accepted before February 1, 1976. Invited papers will deal with evaluation of testicular biopsy; physical, biochemical, and bacteriologic tests of semen; cytochemistry of spermatozoa; histochemistry of male sex organs; ultrastructural localization of enzymes; endocrine profiles and karyotyping of men; clinical examination of infertile men; biochemical and immunologic evaluation of the prostate; postcoital tests; freezing of semen; artificial insemination; and behavioral therapy of male sexual inadequacies. A film entitled "Sexual Therapy of Male Inadequacies" will be shown; there will be a panel discussion on analysis of male "menopause" and erectile and ejaculatory disorders. For information, write to: Dr. E. S. E. Hafez, Department of Gynecology-Obstetrics, 550 E. Canfield, Detroit, Michigan 48201. Telephone: (313) 577-1011. Registration before March 1, 1976, is for a limited number.

Effectiveness of prophylactic Rh immunosuppression after transfusion with D-positive blood

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In a published investigation 42 D-negative men were transfused with single units of D-positive R_1r (CDe/cde) blood to mimic the D-incompatible transfusion accidents occasionally seen in young D-negative women. Twenty-two men received no therapy and 20 were treated with Rh immunoglobulin in a calculated dose of 20 μg of anti-Rh per milliliter of infused red cells. The reported incidence of serologic D alloimmunization was 81.8 per cent in control and zero per cent in treated recipients. Posttransfusion ^{51}Cr clearance studies with a challenge with the use of 5 ml. of D-positive cells from the same donor at five months in these identical subjects vary from the results based upon the presence of circulating anti-D active antibody. Exponential immune clearance curves occurred in two control and nine treated recipients. As the first evidence of D alloimmunization is an immune Cr clearance curve followed at some later time by circulating active anti-D antibodies, these data could suggest that Rh immunization actually was 90.9 per cent in untreated control subjects and 45 per cent in treated men. The dose of passive Rh immunoglobulin effective in prophylaxis of transfusion Rh immunization of a D-negative person is still not definitely established, but apparently it is greater than 20 μg of anti-Rh per milliliter of D-positive erythrocytes received.

CONTINUED REPORTS document the inadvertent transfusion of large volumes of Rh_0 (D)–positive blood to D-negative women of child-bearing age.[1–3] With two recent exceptions[1, 2] it is common knowledge that attempts to suppress primary Rh immunization in such disadvantaged D-negative women have failed.[4, 5] Similar lack of success toward D-immune prophylaxis has been seen in mothers exposed to 100 milliliters or more of D-positive fetal blood by a massive fetomaternal hemorrhage.[6] This dilemma is due chiefly to an uncertainty regarding the exact dose of Rh immunoglobulin to administer per milliliter of circulating D-positive erythrocytes.

Excellent experiments in male D-negative volunteers[7] transfused with small volumes of D-positive red cells (range 11.6 to 37.5 milliliters initially suggested a therapeutic ratio could be derived determining the dosage of anti-Rh passive antibody for the volume of D-incompatible red cells administered. Approximately 20 μg of Rh immunoglobulin to 1 milliliter of D-positive red cells was believed necessary to achieve complete Rh immunosuppression.[7]

This concept was tested subsequently in male subjects receiving much larger volumes of D-incompatible cells, as might occur in these transfusion accidents involving a D-negative woman.[8] Forty-two D-negative volunteers who completed follow-up received single 500 milliliter units of ABO-compatible. D-incompatible R_1r (CDe/cde) blood. Twenty-two were given no immunotherapy, and twenty others were treated with Rh immunoglobulin (Rh_0GAM, Ortho Diagnostics, Raritan, New Jersey) calculated from the optimal ratio of 20 μg of anti-Rh per milliliter of D-positive infused cells. A single nontransfused volunteer was injected with a comparable large amount of Rh immunoglobulin.

Five months after these transfusions, none of the treated volunteers (0/20) exhibited detectable serum anti-D antibodies by several serologic methods. However, 81.8 per cent of the control recipients (18/22) formed active serum anti-D transfusion-induced alloantibodies. Passive anti-Rh was still present in the single control person who was never transfused.

It is the intent of this paper to report ^{51}Cr clearance studies of labeled D-positive red cells given five months

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after transfusion to seven control and 19 of the 20 prophylactically treated recipients of this previous investigation on Rh immunosuppression. Results of the Cr clearance curves suggesting D immunization and the serologic presence of serum anti-D antibodies in this Rh-incompatible transfused population will be compared and interpreted.

Materials and methods

Volunteer subjects. These were the identical population of D-negative men selected with informed consent as previously reported.[8] Two additional healthy D-positive persons, who had never been transfused, also volunteered to receive the same compatible group O,D–positive ^{51}Cr red cells with the posttransfusion challenge studies.

^{51}Cr clearance procedure. The red cell donor was identical for all Cr clearance studies. She was a healthy woman whose red cells had been transfused before without hazard. The serum contained no measured hepatitis-associated antigen (HBAg). Blood groups were O, R_1r (CDe/cde),Ns,P_1,kk.

At the conclusion of the posttransfusion period (five months) each of seven control and 19 treated subjects were injected with 5 ml. of ^{51}Cr packed red cells. Each aliquot was labeled with 50 μc of ^{51}Cr as previously described.[9] The two normal D-positive persons were infused similarly. Except for the initial three subjects listed in Table I, the serum of each recipient was serologically compatible for the ^{51}Cr-treated red cells of the donor.

Equilibration samples for ^{51}Cr radioactivity of the R_1r donor cells were drawn from recipients on Days 1,3,7, and 14 after injection. The aliquots were saved and counted simultaneously for gamma radioactivity. The Cr regression lines were plotted by a method of least squares,[10] and the 100 per cent intercept and the half-life of ^{51}Cr derived mathematically.[9] The mean normal half-life (T/2) of ^{51}Cr for normal random persons by this technique in this laboratory is 27.7 ± 2.6 days.

Serologic techniques. Active anti-D alloantibodies after transfusion were detected with the use of trypsinized screening and panel cells, as has been published.[8]

Results

The data from the seven persons in the control series and the two normal volunteers are found in Table I. The appearance of active anti-D antibody either at the time of the Cr study or 14 days after receipt of the challenge of R_1r radioactive red cells is included. Three of the seven D-negative untreated persons had serologic evidence of Rh immunization before and following the R_1r cells. The Cr curves were typical of exponential immune clearance. The other two untreated subjects (D. K. and O. K.) never demonstrated active anti-D antibody and had Cr clearance times comparable to those of the normal D-positive control subjects (D. H. and R. N.). The mean ^{51}Cr T/2 in these four individuals equalled 25.4 days, which was slightly less than the mean for random normal persons. Two men in the control group (R. S. and J. C.) had no detectable anti-D antibody at either interval in the study, but their Cr clearance times were grossly abnormal.

Table I. Cr clearances and presence of active Rh immunization in untreated male Rh-negative (No. = 7) and Rh-positive control subjects (No. = 2)

Subjects	*T/2 ^{51}Cr (days)*	*Serum anti-Rh_o (D) antibody*	
		Prior to booster R_1r cells	*14 days after booster*
Rh-negative men			
D. F.	4.6	Anti-D + C	Anti-D + C
W. E.	11.1 hr.	Anti-D	Anti-D
D. E.	12.1	Anti-D	Anti-D
R. S.	4.8	None	None
J. C.	12.3	None	None
D. K.*	24.9	None	None
O. K.*	26.2	None	None
Rh-positive controls (never transfused)			
D. H.*	25.1	None	None
R. N.*	25.5	None	None

*Mean normal ^{51}Cr T/2 = 25.3 days.

Table II. Cr clearances and presence of active Rh immunization in Rh-negative men treated with Rh immunoglobulin

Cr clearance	*Subjects*	*T/2 ^{51}Cr (days)*
A. Normal, T/2 > 20 days (No. = 6)	W. M.	20.7
	I. M.	32.4
	R. H.	25.6
	A. K.	23.6
	E. L.	23.5
	N. A.	27.1
B. Early normal or subnormal, T/2 > 20 days or 18 to 20 days with collapse (No. = 4)	W. C.	20.2
	C. H.	21.7
	J. P.	18.0
	R. S.	22.3
C. Abnormal immune, T/2 < 18 days (No. = 9)	J.M.	17.4
	E. A.	9.5
	L. E.	14.0
	R. G.	7.3
	D. H.	4.2
	R. H.	3.1
	R. K.	0.76
	R. L.	9.3
	W. N.	1.9

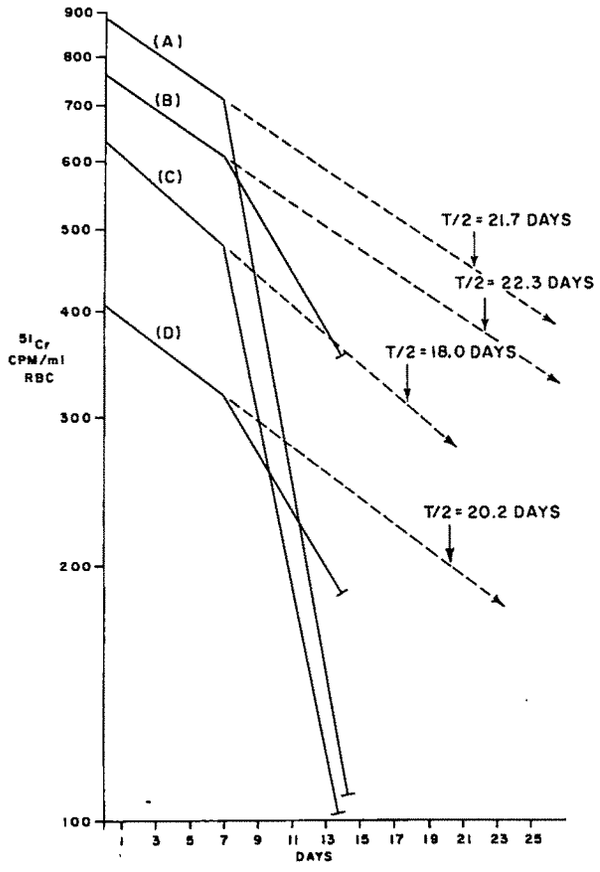


Fig. 1. Cr clearance curves from treated subjects C. H. *(A)*, R. S. *(B)*, J. P. *(C)*, and W. C. *(D)*. The slope of every curve collapses after Day 7.

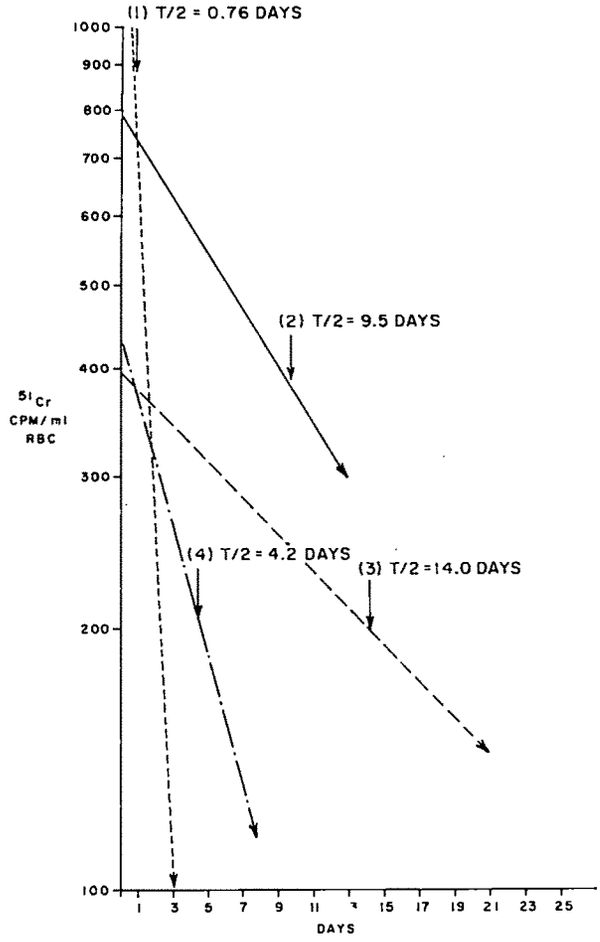


Fig. 2. Cr clearance curves from four treated subjects (R. K., E. A., L. E., D. H.) exhibiting expontential immune cell destruction.

Information on the 19 D-negative men treated prophylactically with Rh immunoglobulin is shown in Table II, as divided into three sections. Since minute amounts of passive Rh immunoglobulin were still found in the nontransfused control subject at five months, an arbitrary criterion for a normal Cr clearance in the treated population was set as a T/2 greater than 20 days. The first section *(A)* contains data of six men believed to have normal Cr clearance. The second *(B)* lists results in four recipients with early normal or just subnormal initial Cr survival curves, all of which exhibited an abrupt collapse between Days 7 and 14. The last section *(C)* contains values of nine subjects whose Cr regression lines were rapid (T/2 less than 18 days) with a single exponential function. By the serologic methods used, no treated recipient exhibited active anti-D alloimmunization then or 14 days after challenge with the R_1r red cells.

The Cr investigations from the four men with biphasic regression "collapse" curves in Section B are illustrated by Fig. 1. Figs. 2 and 3 show Cr elimination curves from eight of the nine volunteers in Section C.

Comment

In the untreated volunteers (Table I) who received experimental R_1r whole blood, the five-month Cr clearance data and serologic presence of active anti-D were at variance in just two recipients (R. S. and J. C.). T/2 Cr values of these two subjects were 4.8 and 12.3 days, respectively. If their rate of exponential disposal of D-positive challenge radioactive cells is due to true Rh immunization even in the absence of serologic anti-D, then the numbers of sensitized transfused untreated D-incompatible volunteers in this experiment would be 20/22, or 90.9 per cent. This figure emphasizes the exceptional risk when a D-negative woman is erroneously given Rh-incompatible blood

and the need to determine the correct dose of passive Rh immunoglobulin.

An even greater disparity exists in the data from the D-negative subjects receiving D-positive whole blood and treated with Rh immunoglobulin. While none of these 20 men formed demonstrable anti-D active antibody,[8] in 13 of them the Cr clearances definitely were unusual.

Twenty-two individuals comprised this original group of treated volunteers, two later being lost to follow-up.[8] Seven days after the injection with passive anti-Rh immunoglobulin in 21/22 recipients some transfused D-positive cells were still noted. At one month after transfusion this incidence decreased to just 8/22.[8] While the rate of immune clearance of the Rh-incompatible segment of the erythron is not the primary factor in preventing D immunization, individuals who exhibit delayed regression curves appear more susceptible to sensitization.[3, 11, 12] This gradual elimination of D-incompatible cells by passive antibody suggests that the encouraging 100 per cent rate of success in Rh immunosuppression reported by serologic data alone may be optimistic.

Six of the 19 treated volunteers had apparently normal Cr elimination curves (range, T/2 20.7 to 32.4 days) as listed in Table II. Four individuals (Group B) had ^{51}Cr curves whose slope was probably normal for the initial seven days, although in one the T/2 value was 18 days. Between Days 7 and 14, in each person there was a uniform rapid negative slope of the regression line. This implied biphasic collapse curves, as illustrated in Fig. 1 as *A, B, C,* and *D.* Although circulating anti-D antibody was then undetected, the similarity and times of these four brisk collapse curves may represent early IgM anti-D production as a primary immune response.

In nine treated recipients (Group C), the ^{51}Cr clearance were rapid and represented by a single exponential curve. Plots from eight of these nine persons are found in Figs. 2 and 3 and can be seen to be characteristic of immune red cell clearance (T/2's 0.76 to 17.4 days). Five of this group formed either active anti-D or anti-CD alloantibodies five months later.

The finding of immune red cell survivals without demonstrable serum antibodies is not new.[13] In many instances no blood group incompatibility exists contrary to that present in this study. Repeating these Cr survival studies with the use of several D-negative–labeled red cells in the 13 volunteers with unusual clearances would have been useful in completely documenting covert D-specific sensitization. Repeated

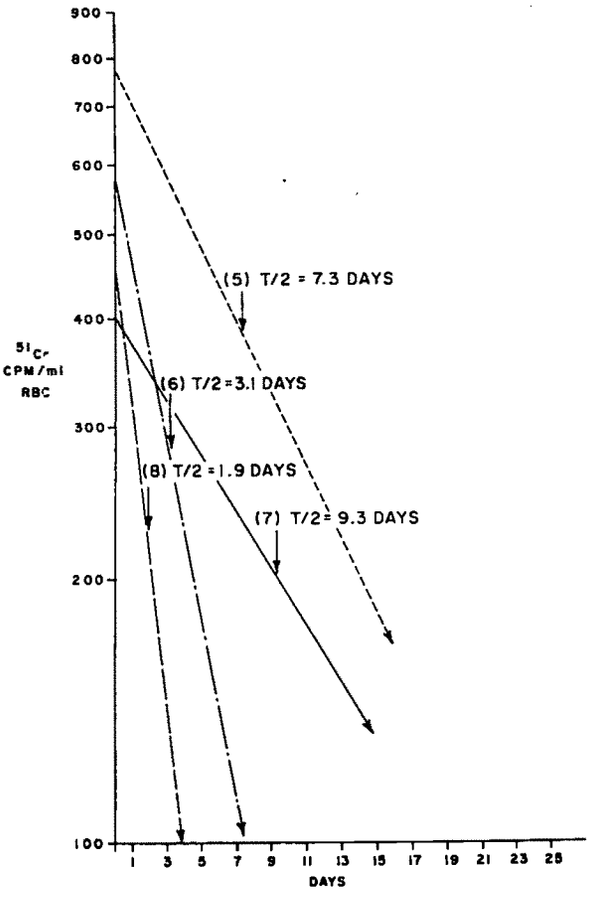


Fig. 3. Cr clearance curves from four treated subjects (R. G., R. H., R. L., W. N.) exhibiting expontential immune cell destruction.

efforts to again recruit these volunteers for this appropriate study became impossible, and they were lost from observation.

Mollison and associates[14] recently investigated Rh-negative subjects injected in varied schedules with 1 milliliter of D-incompatible cells. While approximately 50 per cent became immunized, in some subjects serologic anti-D developed only after several small D-positive stimuli. However, when judged by Cr clearance, Rh immunization was more often apparent after the *initial* injection and *before* the circulating active anti-D alloantibodies appeared. They state ". . . that accelerated clearance of red cells is a more sensitive index for the presence of isoantibodies than any serologic test." Similar findings were reported by Woodrow and associates[3, 12] and by Krevans and co-workers.[15]

From the former investigation it seems reasonable to conclude that the nine treated volunteers (Group C)

received adequate passive immunosuppression preventing circulating anti-D isoantibodies. By the Cr immune clearance rates, however, they would be classed as suspect for Rh immunization. The actual rate of Rh sensitization among treated subjects of this work,[8] judged by these data, could be nine of 20, or 45 per cent rather than 0 per cent.

It is difficult to completely agree with a statement ". . . that administration of approximately 20 μg of anti-Rh_0 (D) per milliliter of Rh-positive red blood cells will suppress the primary immune response to the Rh factor *regardless* of how large the volume of blood . . ."[8] until additional conclusive data become available. Mollison and colleagues[11] concluded that 75 μg of anti-D antibodies inhibited primary immunization by 1 milliliter of Rh_0 (D)–positive red cells. Lower dosages probably would also be effective when Rh-negative subjects are accidently transfused with D-positive blood. Amounts such as 25 μg of anti-Rh per milliliter of red cells have been recently recommended.[5, 16–18]

In instances in which transfusion accidents, especially in D-negative parturient women, were published, apparently successful primary immunization has been achieved when large amounts of Rh immunoglobulin were given. Keith and co-workers[2] used about 36 μg of anti-Rh per milliliter of D-positive red blood cells. Other investigators used 49 μg per milliliter and from 16 to 51 μg of anti-Rh per milliliter of D-incompatible cells,[17] respectively. Only one study[1] involved a subsequent D-positive specific challenge through another D-positive pregnancy which assured that complete Rh immunosuppression was actually accomplished.

Until more data are available involving D-negative persons given volumes of 500 milliliters or more of D-positive blood combined with attempts at passive antibody Rh immunosuppression, a dose between 25 and 35 μg of anti-Rh per milliliter of D-incompatible red cells may prove a useful therapeutic estimate.

I appreciate Dr. William Pollack and his associates providing me with data on these volunteers and permitting the performance of the ^{51}Cr studies. The competence of Mrs. Darlene Tyler (M.T., A.S.C.P.) in radioisotope technique is acknowledged with gratitude.

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Psychometric testing as a basis for counseling patients choosing a method of contraception

A retrospective survey

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To investigate the merits of using psychometric testing as a predictive adjunct to counseling patients in the choice of a contraceptive method, 134 family-planning patients using an oral contraceptive for one year or longer completed the Neuroticism Scale Questionnaire. Clinic records of these patients were examined to determine consistency of contraceptive usage. Patients who were inconsistent in contraceptive usage scored significantly higher on the anxiety factor of the questionnaire. This indicated a greater presence of free-floating anxiety and emotional immaturity for inconsistent users. Results of this study provided support for the findings of two similar studies but failed to provide support for others. However, the over-all predictive ability of the questionnaire scores proved to be low. Further study is recommended.

THE THEORETICAL effectiveness of a contraceptive method in many cases seems unrelated to its effectiveness rate in actual usage. Women who are consciously motivated to prevent the occurrence of a pregnancy choose methods of contraception they are unwilling or unable to use consistently.[1] Social and psychological variables can be as pertinent in the prevention of unwanted pregnancies as the theoretical effectiveness of the contraceptive methods used.[2–5] Studies have shown significant differences in personality traits of consistent and inconsistent contraceptive users as measured psychometrically.[1, 6, 7] Counseling patients in the choice of a contraceptive method could be a more useful process if consistent or inconsistent usage could be predicted by psychometric testing.

Materials and methods

A total of 134 family-planning patients completed the study. These patients were participants in the Louisiana Family Planning Program and had been receiving an oral combination contraceptive pill. The mean age of the study population was 22.9 years (range, 16 to 36). The mean number of years of formal education was 10.8. Twelve of the patients were nulligravid; for the remainder of the population, age at first conception ranged from 13 to 32 years. The mean age at first conception was 17.4 years. The mean number of years the patients had been using oral contraception was 2.4 years. For those patients who had been pregnant, the mean number of pregnancies was 2.0. Fifty-seven of the patients were or had been married and 77 had never been married.

The Neuroticism Scale Questionnaire (NSQ), a brief, 40 item questionnaire, was administered to all subjects of the study.[8] The NSQ is designed to measure bipolar differences in four personality traits which indicate neurotic trends within the normal range. These four factors of the NSQ include (1) sensitivity vs. practicality, (2) depressiveness vs. cheerfulness, (3) submissiveness vs. dominance, and (4) anxiety. The fifth factor of the NSQ is the sum of the four traits measured, or total neuroticism. The higher the standard ten score, the more sensitive, depressive, submissive, anxious, or neurotic the test subject is considered to be.

A patient was considered to be a consistent user of the oral contraceptive if she fulfilled each of the following criteria: (1) kept appointments regularly in a

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Table I. Mean of NSQ scores in standard tens for consistent and inconsistent users with resultant T scores and relative significant difference

NSQ factors	*Mean score, consistent users*	*Mean score, inconsistent users*	*T score, total population*	*Significance (2P) ≤0.05 level of confidence*
Sensitivity vs. practicality	3.988	3.820	0.491	No significant difference
Depressiveness vs. cheerfulness	5.750	5.560	0.519	No significant difference
Submissiveness vs. dominance	5.773	6.000	0.597	No significant difference
Anxiety	6.750	7.520	2.121	Significantly increased for inconsistent users
Total neuroticism	5.619	6.040	1.169	No significant difference

Table II. Number and percent of standard ten scores for NSQ components for inconsistent users (N = 50)

	Components of NSQ									
	Sensitivity		*Depression*		*Submissiveness*		*Anxiety*		*Total neuroticism*	
Sten scores	*Number*	*%*	*Number*	*%*	*Number*	*%*	*Number*	*%*	*Number*	*%*
10*	—	—	1	2.0	2	4.0	9	18.0	2	4.0
9	1	2.0	4	8.0	8	16.0	14	28.0	5	10.0
8	—	—	5	10.0	4	8.0	2	4.0	5	10.0
7	2	4.0	3	6.0	7	14.0	10	20.0	10	20.0
6	4	8.0	11	22.0	6	12.0	7	14.0	9	18.0
5	13	26.0	14	28.0	8	16.0	4	8.0	5	10.0
4	11	22.0	4	8.0	8	16.0	2	4.0	8	16.0
3	6	12.0	5	10.0	6	12.0	1	2.0	4	8.0
2	4	8.0	1	2.0	—	—	—	—	2	4.0
1†	9	18.0	2	4.0	1	2.0	1	2.0	—	—
	Practicality		Cheerfulness		Dominance		Not anxious		Low neuroticism	

*High.
†Low.

manner assuring her a constant supply of medication; (2) pill count was correct when patient kept supply appointments; (3) she had not been pregnant while using the oral contraceptive method. A patient was considered to be inconsistent if she was deficient in any one of the following criteria: (1) failed to keep appointments regularly enough to prevent running out of pills; (2) wrong pill count without a satisfactory reason; (3) she became pregnant while on the oral contraceptive pill.

This patient population was carefully selected to eliminate those patients who were lost to follow-up or changed methods before the completion of one full year. We believe one year of objective data is needed to establish a pattern of consistent or inconsistent usage for this method.

Results

A significant difference in personality traits of consistent and inconsistent contraceptive users was found for only one of the NSQ factors. Anxiety was shown to be increased significantly ($P \leq 0.05$) for the inconsistent contraceptive users. The mean scores for depressiveness vs. cheerfulness, submissiveness vs. dominance, and total neuroticism were not statistically significant. The values of the means of the standard ten scores on the five factors of the NSQ for consistent and inconsistent users with the resultant T scores are shown in Table I. For the total study population, the mean of the standard ten scores for sensitivity vs. practicality was lower than the average range indicating all of the patients leaned more toward tough practicality rather than sensitivity. The actual number of inconsistent patients with their standard ten scores and per cent of total for each factor tested is found in Table II. The same information for consistent patients is found in Table III.

Comment

An inverse relationship exists between the findings of this study and the findings of Kane and associates[6] for postpartum patients in North Carolina. In the North Carolina study, significant differences were found for all factors of the NSQ with the exception of

Table III. Number and percent of standard ten scores for NSQ components for consistent users (N = 84)

	Components of NSQ									
	Sensitivity		*Depression*		*Submissiveness*		*Anxiety*		*Total neuroticism*	
Sten scores	*Number*	*%*	*Number*	*%*	*Number*	*%*	*Number*	*%*	*Number*	*%*
10*	—	—	3	4.0	1	1.0	6	7.0	1	1.0
9	2	2.0	5	6.0	8	10.0	11	13.0	7	8.0
8	2	2.0	7	8.0	5	6.0	10	12.0	6	7.0
7	5	6.0	14	17.0	21	25.0	22	26.0	17	20.0
6	5	6.0	23	27.0	11	13.0	18	21.0	13	15.0
5	23	27.0	8	10.0	15	18.0	6	7.0	19	23.0
4	14	17.0	10	12.0	6	7.0	5	6.0	9	11.0
3	12	14.0	10	12.0	17	20.0	5	6.0	8	10.0
2	8	10.0	2	2.0	—	—	—	—	4	5.0
1	13	15.0	2	2.0	—	—	1	1.0	—	—
	Practicality		Cheerfulness		Dominance		Not anxious		Low neuroticism	

*High.
†Low.

anxiety, for which no significant difference was shown. The findings of Kane in a study of motivational factors of abortion patients did show a significant increase in anxiety scores of patients seeking abortion as contrasted to the scores of a nonpregnant group. Kane discounted this finding because a higher level of anxiety was expected in patients awaiting abortion.

Emotional immaturity is one of the dimensions measured by the anxiety scale of the NSQ. Bakker and Dightman[1] found pill-forgetting women possessed a "considerable degree of immaturity." Thus, the result of this study seems to lend support to the findings of Bakker and Dightman.

The intent of this study was to administer a standard NSQ to a family-planning patient population in an effort to uncover a patient profile which would correlate with the continuation or lack of continuation of a contraceptive method. The only factor which appears to have any statistically significant relationship is anxiety, which was significantly increased in inconsistent users. Since anxiety seems to be the key factor in this particular study, perhaps the questions relating to the scoring of anxiety on the questionnaire could be extracted which would make testing a simpler process in a large population. The predictability of a patient's consistent and effective continuation of a contraceptive method would be valuable in counseling patients choosing a method of contraception. If inconsistence of usage were predicted, perhaps it could be suggested that the patient choose a method which would require less individual motivation. Certainly women with medical conditions which make them at high risk for pregnancy would benefit if one could predict that a selected method would have its best continuation rate. We believe further studies into developing tools able to uncover certain motivational factors in patient populations will prove beneficial. A prospective study of this kind on a larger scale needs to be done to validate our findings in this small retrospective study.

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Amenorrhea following oral contraception

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A prospective study was done on 326 nulliparous women who ceased oral contraception. Follow-up was obtained on 311. The mean age was 20.3 years (S.D. 2.5). Eighty-nine per cent began menstruating within 60 days after stopping therapy, and only 7 took 180 days or longer to menstruate. Late menarche strongly correlated with post-pill amenorrhea (PPA), but continous length of time on oral contraceptives and type of oral contraceptive showed no significant relationship. The incidence of PPA was 2.2 per cent. All women did eventually menstruate spontaneously, the longest period of amenorrhea being 540 days.

WOMEN ELECTING oral contraception as a method of choice for pregnancy prevention are frequently young and nulliparous. Post-pill amenorrhea (PPA) may be a threat to future conception. Amenorrhea following oral contraception has been well documented, but the predisposing factors which correlate with this syndrome have not been well defined. Since most papers discussing the problem of PPA are retrospective studies, the precise incidence is difficult to determine. Larsson-Cohn[1] described four cases of amenorrhea over 6 months' duration in 516 women who were on oral contraceptives. Golditch[2] has estimated the incidence of PPA to be 2.2 per 1,000 users. In a study of 210 women with secondary amenorrhea of 6 months' duration 30 per cent were PPA cases,[3] and in another series of 439 cases of secondary amenorrhea, 9.3 per cent occurred after the cessation of oral contraceptives.[2] Most authors have defined PPA as amenorrhea of 6 months or more after the cessation of oral contraceptives. Irregular menses prior to the institution of oral contraceptive therapy,[4] late onset of menarche,[5] length of time on oral contraceptive agents, and the use of combined oral contraceptives have all been suggested as predisposing factors. Table I shows the experience of previous authors on the relationship of prior irregular menses to PPA.

Late menarche was implicated as a predisposing factor by Grant,[6] and Steele and associates[3] noted menarche at age 16 or older in 8 of 132 women with PPA. Golditch[2] and Shearman[7] stated that PPA is in no way correlated with the length of time the woman has been on oral contraceptive agents and Goldfarb[8] recommended that women in the childbearing age be allowed to have a spontaneous menses after being on the pill 2 or 3 years.

PPA was found only after the use of combined oral contraceptive agents by MacLeod and associates[9] and Starup.[10] Other authors have reported amenorrhea after sequential pills with much less frequency.[2, 7] This phenomenon probably is an artifact produced by virtue of the fact that many more women are on combined pills than sequential pills.

In the attempt to delineate the parameters which may predispose to PPA of 6 months' or more duration a prospective study was done on 326 women at Brown University.

Material and methods

A total of 326 nulliparous women were studied from August 1, 1971, to August 1, 1974. All women who requested oral contraception, and who had no medical contraindication, were included in the study providing that they had not been on oral contraceptives previously. Follow-up was obtained on 311 cases and 15 were lost to study. Of the 311, the age range was from 16 to 38 years of age, with a mean of 20.3 years (S.D. 2.5). History of the patient's menses during the year prior to

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going on oral contraceptive agents was obtained. The mean number of days between menstrual periods was calculated and the variance of the cycle was considered one half the number of days of the difference between the longest and shortest cycle in the last year. PPA was defined as a lack of spontaneous menses for 180 days or more after ceasing oral contraceptive medication. The group was somewhat selective since any woman having six or fewer periods per year or having a period of amenorrhea of longer than 165 days was not placed on oral contraceptive medication. Ninety-eight per cent of the patients had not been amenorrheic for longer than 60 days in the year prior to oral contraception.

Results

Only 11 patients took 120 to 179 days to menstruate and seven began their post-pill menstrual period after 179 days. Table II depicts the time of onset of menses after oral contraception. Eighty-nine per cent began menstruating within 60 days after pill therapy. Women with PPA of 180 days or greater began menstruating at 180, 206, 240, 330, 345, 450, and 540 days, respectively. The number of months any patient was on the pill continuously before stopping oral contraception showed no correlation with the time of onset of first menses. Of the seven patients with PPA, three patients had their menarche at age 16 years. Seven of the 311 patients were on sequential contraceptives and none was amenorrheic longer than 65 days after pill therapy. A total of 153 (49 per cent) menstruated within their mean cycle plus their normal variance. All 311 patients did eventually menstruate spontaneously.

Comment

An incidence of PPA of 2.2 per 100 in this study reflects a tenfold increase to the estimate by Golditch.[2] Most students of PPA have incriminated previous irregular menses as a predisposing factor. The present study does not confirm this opinion. This study, however, is biased in two ways. (1) No woman was placed on oral contraceptive therapy unless her cycle was reasonably regular (as defined in Materials and Methods). (2) The authors considered only the year prior to the beginning of oral contraceptive agents in determining regularity of the cycle. No doubt, if a past menstrual history dating back to menarche was obtained, a much higher incidence of menstrual irregularity would have been discovered. In the present study, menstrual irregularity in the year prior to institution of oral contraception showed no correlation with PPA. Only 11 patients took 3 to 6 months to begin menstruating and all of these in the year prior to therapy had a cycle plus variance of 42 days or less. Also the seven patients with PPA of 6 months or greater all had a cycle plus variance of 42 days or less.

Table I. PPA patients with prior irregular menses

Ref. No.	*Prior irregular menses/cases of PPA*	*PPA with prior irregular menses (%)*
7	16/69	23
3		37
10	17/31	55
6	33/68	48
11		54
2	17/41	41

Table II. Relative frequency, onset first menses after oral contraception

Days	*No.*	*Frequency (%)*
20 to 29	94	30.3
30 to 59	183	58.9
60 to 89	11	3.5
90 to 119	5	1.6
120 to 149	6	1.9
150 to 179	5	1.6
180 ≥	7	2.2
Total	311	100

The four patients who were amenorrheic for 9 months or longer had studies performed to exclude other endocrinopathies. Skull x-rays, visual field examination, T3, T4, 17-ketosteroid, and 17-hydroxycorticoid studies were undertaken and found to be within normal limits.

Medroxyprogesterone acetate, 10 mg. daily for 6 days, was administered orally to five of the seven patients with PPA (the patients who were amenorrheic 180, 330, 345, 450, and 540 days). Withdrawal bleeding was induced in four patients (180, 345, 450, and 540 days). It was suggested to the woman who did not exhibit progesterone withdrawal bleeding that she have a serum FSH and LH assay but she refused. It is interesting that the patient with the longest period of PPA (540 days) did bleed after progesterone withdrawal 3 months after oral contraceptive therapy but did not have progesterone withdrawal bleeding after 12 months of PPA. At this time Premarin, 2.5 mg. daily orally for 20 days, with medroxyprogesterone acetate, 10 mg. daily orally on the last 6 days of the Premarin therapy, was used to induce bleeding. On the fifth day of bleeding clomiphene citrate, 50 mg. daily, was administrated orally for 5 days. Her basal temperature remained flat and she did not menstruate. Although she was not apparently under weight, her menses did

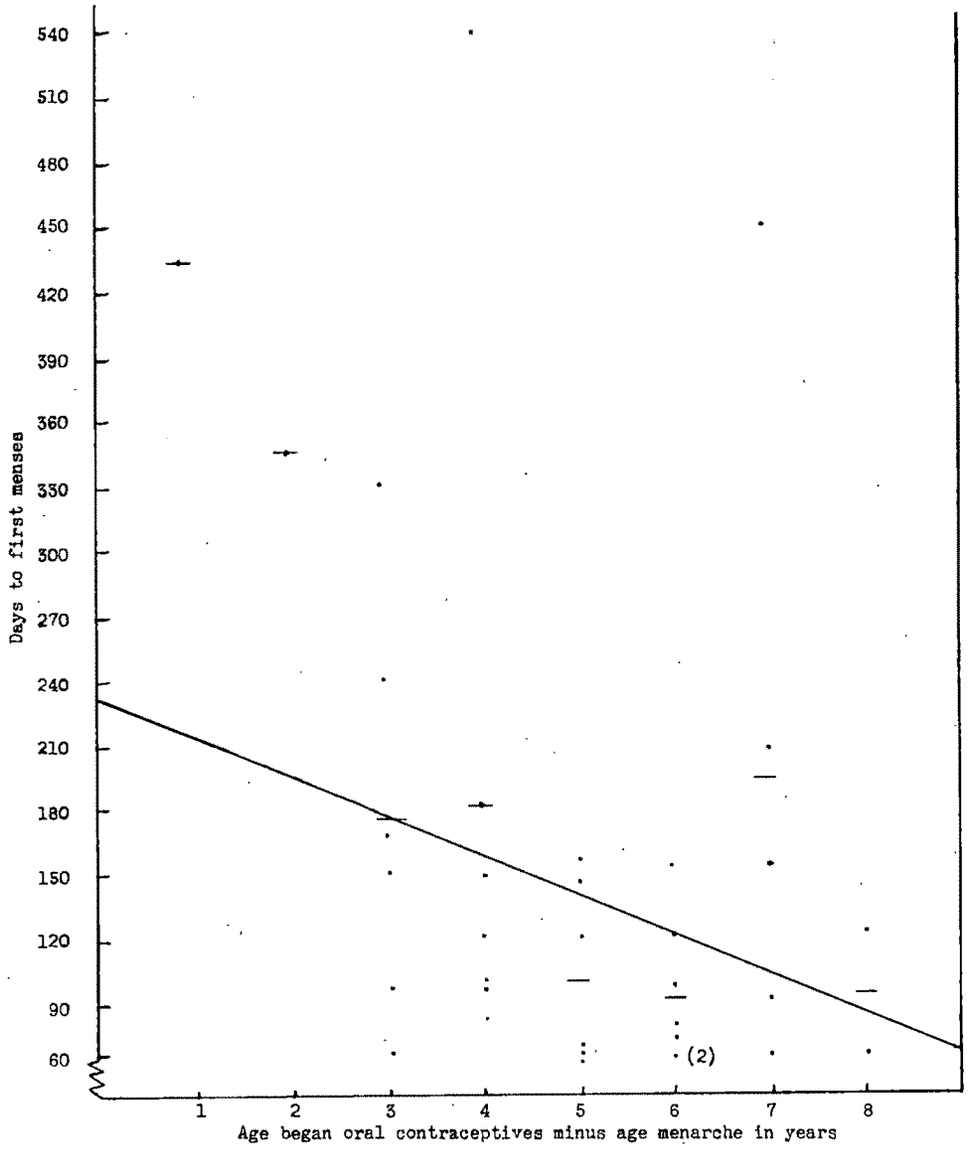


Fig. 1. Relationship of days to first menses to years between menarche and age at which OC were begun. Pearson's regression coefficient = −16.73. (2) = Two values at same position. − Indicates mean value.

resume spontaneously at 540 days after she had gained 10 pounds, and her menses did remain regular.

Late menarche (16 years) was associated with PPA. Only 12 patients had their menarche at age 16 (none older than 16) and three of these had PPA compared to four of the remaining 299 patients ($P < 0.001$).

The high correlation of late menarche with PPA suggests another predisposing factor for PPA. Should oral contraceptive therapy be instituted close to the menarche, while the hypothalamic-pituitary-ovarian axis is still in a fragile state of equilibrium, PPA might result. To test this hypothesis the age at which oral contraception was begun minus the age of menarche was calculated in all patients who took 60 days or longer to begin menstruation after pill therapy. Indeed, the less the number of years between menarche and beginning the oral contraceptives the longer it took those patients to menstruate. Fig. 1 is a scattergram depicting the age at which the oral contraceptive was instituted minus the age of menarche and the time of onset of menses in patients who took 50 days or longer to menstruate after oral contraceptive

therapy. Although the regression line shows a negative correlation between the years between menarche and onset of oral contraceptives and length of time to resume menses after stopping therapy, the numbers are too small to be statistically significant. Obviously, this relationship would apply only when there is not a great interval between menarche and beginning of oral contraceptive therapy.

Several authors have suggested that PPA occurs only after combined pills.[9, 10] We could not test this theory since only seven patients were on sequential pills and none was amenorrheic. Other authors report a lesser incidence of PPA on sequential pills. Two explanations for this phenomenon must be considered. It is true that the progestational agents in oral contraceptives are potent LH suppressors and on a theoretical basis sequential pills may produce less suppression than combination pills. However, marketing figures in the United States for the years 1971 through 1973 document that sequential pills accounted for only 6 per cent of the total oral contraceptive market.[12] The infrequency of PPA after sequential pills could therefore be due to less frequent use of this type of agent.

Length of continuous time on oral contraceptives revealed no correlation to PPA. For the entire group, the range of time on the pill was from 1 to 84 months, with a mean of 11.2 months (S.D. 9.29). In those women with PPA, the range was from 7 to 18 months, with a mean of 9.7 months (S.D. 3.9). As a group the women of PPA were on oral contraceptive agents fewer continuous months than the entire group.

Conclusions

In these 311 patients the incidence of PPA was 2.2 per cent. Menarche at age 16 appeared to be a predisposing factor and the closer a woman was to her menarche when she commenced therapy the longer it took her to re-establish her menstrual cycle. The number of continuous months on oral contraceptives does not appear to be correlated with PPA.

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Symposium on Medical Genetics

The Hungarian Society of Human Genetics will present an international symposium, "Symposium on Medical Genetics," with World Health Organization support in Debrecen-Hajdúszoboszló, Hungary, on April 27-29, 1976. Topics of the Symposium include: (1) Cytogenetics, (2) Population Genetics, (3) Prenatal Diagnosis and Genetic Counseling, (4) Chromosomal Mapping, (5) Clinical Genetics on the Molecular Level—Hemoglobinopathies and Immunogenetics. Further inquiries should be addressed to: Human Genetics Laboratory, H-4012 Debrecen, P. O. Box 37, Hungary.

Infertility following wedge resection of the ovaries

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Seven cases of polycystic ovarian disease were investigated by laparoscopy and endocrinologic tests after failure of ovarian resection to restore fertility. One case of bilateral and two cases of unilateral ovarian atrophy were recorded. In all seven patients the common features were extensive periovarian and peritubal adhesions. Four of the six patients amenable to cure were submitted to reconstructive surgery; three of them conceived and had normal deliveries. The present observations support the plea to relegate the surgical approach to a minor position in patients with Stein-Leventhal syndrome and stress the importance of meticulous surgical technique in the performance of ovarian surgery. Laparoscopic investigation is mandatory in all cases of unsuccessful ovarian resection.

DESPITE the growing interest in conservative methods for induction of ovulation in polycystic ovarian disease, wedge resection of the ovaries is still very much in use. Its beneficial effects are well documented.

According to Goldzieher's[1] survey of published results, regular cycles follow surgery in 80 per cent of patients and desired pregnancy is achieved in 63 per cent. In some series, however, less than 10 per cent of subjects experienced regulation of menses and only 13 per cent conceived.[2] It is generally agreed that fertility is restored less often than the menstrual rhythm.[1, 3] Questionable diagnosis and surgical complications may be involved in the failure of ovarian wedge resection to achieve its aim.

The present report describes a series of seven cases of polycystic ovarian disease submitted to laparoscopic and endocrinologic investigations after the failure of surgery to restore fertility.

Material and methods

During a 6 year period (1967-1972) 11 women, who had previously been submitted to wedge resection of the ovaries in other hospitals with diagnosis of Stein-Leventhal syndrome, were investigated. Their complaint was persistent sterility, with or without obvious menstrual disorders. Four of them, whose operation records showed that at operation other pathology, such as malformations, tumors, or sequelae of pelvic inflammatory disease, was found beside polycystic ovaries, were not included in the present series in spite of similar findings.

Six of the seven patients of the series were 26 to 28 years of age and one was 39. Ovarian surgery had been performed 2 to 9 years previously. All the patients had been treated, after endocrine studies were carried out before and after surgery, in different institutions and by private practitioners. As reliable data were not available in most cases, however, only information known to the patient and surgical records were used in establishing the early history.

The investigations performed included the following. (1) Endocrine evaluation, including 17-ketosteroid excretion and their fractionation, adrenocortical suppression and ovarian stimulation (dexamethasone-HCG test according to Jayle[4]) plasma LH studies under basic conditions and after clomiphene administration, estrogen excretion, evaluation of thyroid function. (2) Hysterosalpingography with water-soluble contrast medium. (3) Laparoscopy, generally within 2 to 4 days after occurrence of the thermal shift in clomiphene-treated cycles.

Case reports

Case 1. A. B., 27 years of age, sought advice for secondary sterility and oligomenorrhea. Following

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menarche at 13, she had normal periods for 3 days' duration every 30 days up to the age of 21. Married at 17, she had at 19 a normal pregnancy and delivery. For the last 6 years she menstruated for 10 days every 45 to 60 days and she could not conceive. In 1965, at the age of 25, following culdoscopic examination which revealed enlarged, white, smooth ovaries, she was submitted to bilateral ovarian resection. The postoperative course was complicated by fever and abdominal pains. The menstrual rhythm remained unchanged and no conception followed.

On examination, no physical abnormalities were observed. The endocrine findings were within normal limits. At laparoscopy, abundant pelvic peritoneal adhesions were found. The left adnexae were included in a single fibrotic mass. The right ovary was slightly enlarged, micropolycystic, and partially covered by the adherent, distorted tube.

The patient was operated on again in 1967. The left ovary was reduced to a fibrotic band. The right ovary was freed from its extensive adhesions to the tube, the posterior wall of the uterus, and broad ligament. The peritoneal lining of the right tube was carefully reconstructed.

The postoperative course was uneventful. One year later the patient conceived under clomiphene treatment (500 mg. per cycle, five cycles) and had a normal term delivery.

Case 2. S. M., 28 years of age, had been married 8 years when first seen. Since menarche at the age of 10, she had menstruated 4 to 5 days every 1 to 4 months. For that reason she had been submitted to bilateral ovarian resection at the age of 19, shortly before marriage, in 1962.

After operation she had normal menses for 3 years, followed by recurrence of oligomenorrhea. She was never pregnant. On physical examination, no endocrine stigmas were recorded. The only positive finding at endocrine evaluation was hypothyroidism. Her LH plasma values were 20 to 24 mU per milliliter. Under thyroxine treatment (100 μg per day) her periods became regular, but were apparently anovulatory. At laparoscopy, a few months later, dense peritubal and periovarian adhesions were seen. The patient underwent reconstructive surgery in December, 1971. Both ovaries were enlarged and polycystic, almost completely covered by adhesions involving omentum and tubes. The distal part of the left tube was completely buried by adhesions deep into the pelvis. The left tube was only partially covered by adhesions, but the fimbriae were almost completely adherent to the ovarian surface.

Following careful reconstruction and endocrine treatment which included thyroxine (100 μg per day) and clomiphene therapy (750 mg. per cycle), the patient conceived after the third clomiphene course and delivered at term in April, 1973. She delivered again in November, 1974.

Case 3. D. Y., 39 years of age, had been married 19 years when first seen. She had been amenorrheic since the age of 16, after 1 year of almost regular menstruations, and was never pregnant. She was investigated and treated in several hospitals up to the age of 33, when wedge resection of the ovaries was performed in 1965. After operation she remained amenorrheic, but under HMG (Pergonal) therapy she menstruated and apparently had ovulatory cycles. No pregnancy, however, followed.

On examination, we found a normally developed woman, without acne or hirsutism. Excretion of 17-ketosteroids was in the upper range (11 to 14 mg. per 24 hours), with a high excretion of dehydroepiandrosterone (1.6 mg. per 24 hours). Excretion of estrogens was very low (6.4 μg per 24 hours). Repeated LH plasma estimations gave values of 20 to 22 mU per milliliter.

Dexamethasone suppression, followed by ovarian stimulation according to Jayle,[4] seemed to establish heightened adrenal androgenic function. At laparoscopy, the right ovary appeared as a thick fibrotic band with the tip of the appendix attached. The left ovary was small and atrophic, adherent to the uterus. Uterus and tubes were normal. As ovarian resection had resulted in a condition very close to surgical castration, the patient was advised that further treatment was futile.

Case 4. S. H., 26 years of age, had been married 8 years when she consulted us because of primary sterility. Since menarche at the age of 15, she had suffered from oligomenorrhea, with periods of 6 days' duration every 2 to 3 months. In 1965, at the age of 19, shortly after marriage, she was submitted to bilateral ovarian resection. The menses became more regular, lasting 3 to 5 days every 29 to 35 days, but conception did not occur. Under clomiphene treatment she had apparently ovulatory cycles. Three years previously, hysterosalpingography demonstrated normal tubal patency.

On examination we found a normally developed woman, with abundant acne, no hirsutism. The BBT curve was biphasic, with a short luteal phase. Endocrine work-up did not reveal any abnormality. 17-Ketosteroid excretion and fractionation findings were well within normal limits. Serial daily LH plasma estimations showed values of 23 to 25 mU. per milliliter and a peak of 84 mU. per milliliter. Early luteal laparoscopy demonstrated a normal uterus, a right ovary covered by adhesions joining the sygmoid to the broad ligament. The distal part of both tubes was covered with adhesions. Tubal patency was preserved. No signs of ovulation were seen.

The patient was operated on again in January, 1973. The dense peritoneal adhesions were carefully dissected and ovaries and tubes freed. A remarkable finding was that the adhesions on both ovaries converged to a linear scar, the suture line of the wedge resection.

Table I. Pre- and postoperative menstrual pattern, and relevant postoperative findings in seven infertile women

Serial No.	*Presenting Age*	*Age at wedge resection*	*Preoperative symptoms*	*Postoperative course*	*Postoperative menstrual function*
1. A.B.	27	25	Oligomenorrhea 6 yr. dur. Sec. sterility. No hirsutism, no acne	Fever, abdominal pains	Oligomenorrhea, anovulation
2. S.M.	28	19	Oligomenorrhea 9 yr. No hirsutism, no acne	Normal	Regular 3 yr. then oligomenorrhea
3. D.Y.	39	33	Sec. amenorrhea of 17 yr. dur. Primary sterility. No hirsutism	Normal	Amenorrhea. Normal cycles under Pergonal treatment
4. S.H.	26	19	Oligomenorrhea of 4 yr. dur. No conception during 1 yr. Acne, no hirsutism	Normal	Almost regular, but anovulatory cycles
5. M.E.	28	25	Oligomenorrhea 5 yr., then amenorrhea, 7 yr. Primary sterility. No hirsutism	Normal	Amenorrhea. Satisfactory reaction to induction of ovulation with clomiphene or Pergonal
6. A.Z.	27	24	Regular periods, anovulation. Primary sterility. No hirsutism	Normal	Regular, ovulatory cycles
7. W.S.	28	21	Oligomenorrhea of 8 yr. dur. Primary sterility. Hirsutism, marked acne	Normal	Regular for a few months, then oligomenorrhea

Following operation, the woman was treated for several months with clomiphene (750 mg. per cycle). The cycles were apparently normal but the luteal phase remained short. A trial with prednisone (5 mg. per day) was successful and the patient conceived in the second month of prednisone therapy. She was delivered at term by cesarean section in May, 1974.

Case 5. M. E., 28 years of age, was first seen after 8 years of marriage. Following menarche at the age of 13 and up to the age of 18 she had menstrual periods of 8 to 12 days' duration every 2 to 4 months, then she became amenorrheic. Before and after marriage she was treated with prednisone, later with clomiphene, and then with HMG (Pergonal). Under clomiphene or Pergonal therapy she had apparently normal cycles but did not conceive. Conservative treatment was then abandoned and wedge resection performed at the age of 25, in 1970. As the menstrual rhythm remained unchanged, the patient was treated again with clomiphene and HCG and her periods became more regular.

On admission, 3 years later, no physical abnormality was observed. Endocrine work-up was uncontributory. Following clomiphene, the patient had a normal menstruation, with typical BBT curve and elevated urinary excretion of pregnanediol. LH plasma estimations in consecutive days were 23, 25, 26, 48, and 72 mU. per milliliter, and FSH plasma estimations in the same days were 91, 84, 93, and 130 mU. per milliliter. At laparoscopy the ovaries were found to be almost completely covered by thick adhesions involving both tubes and intestine loops. The patient has been advised that reconstructive surgery is mandatory in her present condition, but up to now has refused operation.

Case 6. A. Z., 27 years of age, married 9 years, consulted us for primary sterility. Since the age of 13 she had regular periods of 4 days, every 28 days. After 1 year of marriage without conception, she sought medical advice and was treated with clomiphene for long periods. In 1970, at the age of 24, shortly after a laparoscopic examination, she was submitted to ovarian resection. Following operation her periods remained regular but the desired pregnancy was not achieved.

When examined, she had a biphasic BBT curve, normal LH plasma values with an ovulatory peak of 81 mU. per milliliter. At laparoscopy, a very small, micropolycystic, nonfunctioning ovary and a normal tube were seen on the right side. On the left, a normal ovary with a hemorrhagic corpus luteum was joined by adhesions to a patent tube, the fimbrial part of which was included in transparent membranes. The pouch of Douglas was obliterated by adhesions. Reconstructive surgery has been advised.

Case 7. W. S., 28 years of age, had been married 10 years when first seen because of primary sterility. Since the age of 13 she had scanty menstrual periods every 2 to 4 months. In 1968, at the age of 21, she had been submitted to wedge resection of the ovaries. Her periods became regular and remained so for several

submitted to wedge resection of ovaries

Laparoscopic findings	*Endocrine assessment*	*Treatment*		*Results*
		Surgical	*Medical*	
Peritoneal adhesions, severe. Atrophy of left ovary. R. polycystic ovary. R. periovarian and peritubal adhesions	Within normal limits	Removal of periovarian and peritubal adhesions	Clomiphene	Conception and normal delivery
Severe peritubal and periovarian adhesions. Enlarged polycystic ovaries	Hypothyroidism	Removal of periovarian and peritubal adhesions. Terminal tuboplasty	L-Thyroxine	Conception and normal delivery
Atrophy of R. ovary. Ovarian appendicular adhesion. Small L. ovary adherent to post. leaf of broad ligament. Normal tubes	Increased androgenic function, probably of adrenal origin	—	—	Patient dismissed
Severe periovarian and peritubal adhesions. Tubes patent	Within normal limits	Lysis of periovarian and peritubal adhesions	Prednisone	Conception and normal delivery
Ovaries covered by thick adhesions involving tubes and intestine	Within normal limits. LH peak after clomiphene	Proposed, patient reluctant	—	—
R. atrophic ovary. L. ovary normal with hemorrhagic corpus luteum. Left peritubal adhesions	Normal	Proposed	—	—
Bilateral severe peritubal and periovarian adhesions	Increased androgenic function, probably of adrenal origin	Lysis of periovarian and peritubal adhesions, (Dec. 1973)	Dexasone	Apparently ovulatory cycles

months, then oligohypomenorrhea recurred. Since puberty she had suffered from acne, especially on the face.

Physical examination revealed a fat woman with feminine habitus, normally developed breasts without galactorrhea, marked hirsutism on the face, lips, neck, and buttocks, and a male-type pubic escutcheon. Her external genitalia were normal, the uterus was of normal size, and the ovaries did not appear enlarged.

The relevant endocrinologic findings were upper-normal values of 17-ketosteroid excretion (10.8 to 15.2 mg. per 24 hours) with high levels of androsterone (5.6 to 6.1 mg. per 24 hours) and dehydroepiandrosterone (1.8 to 3.0 mg. per 24 hours) on fractionation. Those high levels were reduced to within the normal range (1.9 and 0.8 mg. per 24 hours, respectively) under dexamethasone suppression, and remained normal under HCG stimulation. The total estrogen excretion was 7 to 15.7 μg per 24 hours. LH plasma estimations gave values of 14 to 16 mU. per milliliter.

At laparoscopy, the left adnexae were found to be buried in a mass of thick adhesions. Peritubal and periovarian adhesions were present in lesser measure on the right side, where the ovary was polycystic, partially covered by the adherent ampullar part of the patent tube.

The patient was classfied as having a mild case of adrenal androgenic hyperactivity. In December, 1973, she was submitted to reconstructive pelvic surgery. Under maintenance dexamethasone therapy she has regular, apparently ovulatory cycles. Her husband is currently under treatment for oligospermia.

Comment

Relevant data on the seven cases of unsuccesful ovarian resection described here are presented in Table I.

A retrospective evaluation of the conditions which at different times and in different places prompted the abandonment of conservative therapy and the report to surgery is obviously preposterous and will not be pursued. Even in Case 2, where hypothyroidism was proved 9 years after surgery, the assumption that thyroid dysfunction caused the polycystic ovarian disease seems unjustified, as the patient had regular menses for 3 years following operation. It may be relevant, however, to note that, with the exception of Case 7, none of those patients had hirsutism, a prominent sign in the Stein-Leventhal syndrome.

The common features in the present series are extensive periovarian and peritubal adhesions. A stormy postoperative course easily explains their presence in Case 1. All other cases were asymptomatic.

Similar cases have been reported occasionally in the literature. Stein,[5] when reoperating six women for suspected recurrence of the polycystic ovarian disease, found dense peri-ovarian adhesions to omentum and/or bowel in four of them. Kistner[6] was impressed

with the number of patients having recurrent amenorrhea or oligoovulation subsequent to wedge resection in whom extensive peritubal adhesions were demonstrated by culdoscopy. Cohen[7] studied a number of women who had relatively regular, apparently ovulatory cycles after ovarian resection, but remained infertile, and found endoscopic evidence of ovarian adhesions extensive enough to prevent ovulation. Goldzieher[1] assumed that surgical factors may partly explain why fertility is corrected by wedge resection less often than the menstrual rhythm. The increasing use of gynecologic endoscopy, and particularly of laparoscopy, is now proving the important role of pelvic peritoneal adhesions in limiting the success of wedge resection.[7]

The present series of cases shows that the surgical procedure alone may be responsible for the persistence of infertility through a mechanical factor which makes pregnancy virtually impossible. Adequate reconstructive surgery becomes a precondition to successful fertilization in those cases where ovarian resection cured anovulation, and to successful fertilization following induction of ovulation by clomiphene or other agents in those cases where ovarian surgery failed to correct anovulation. This policy has been applied in four of the six cases where reconstructive surgery was possible, with gratifying results.

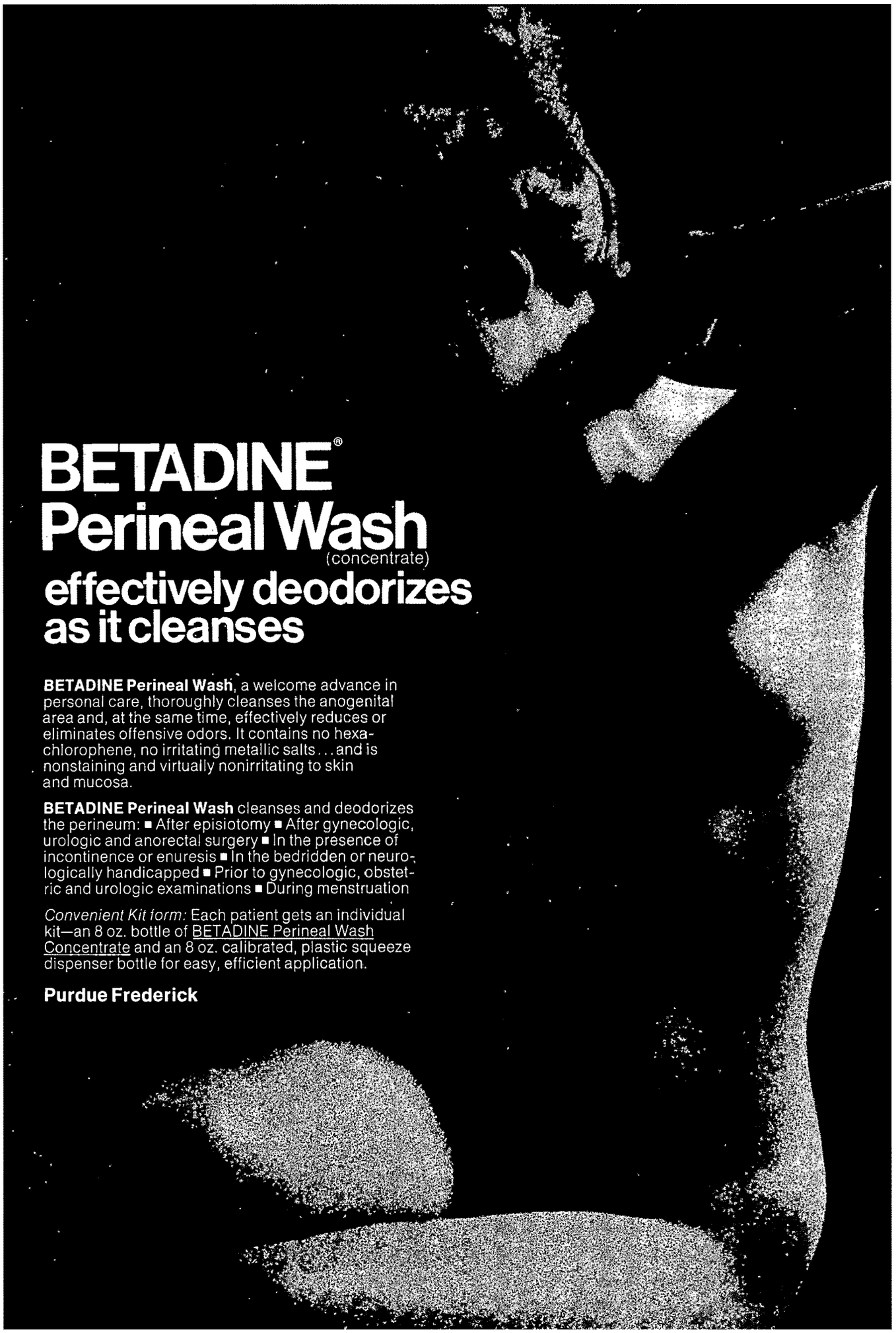
While it is impossible to establish at present the frequency of periovarian and peritubal adhesions following ovarian resection, it may be safely assumed that they constitute one of the important causes of failure of the surgical procedure to achieve its ends. Laparoscopy is therefore mandatory in the investigation of the unsuccesful results of bilateral ovarian resection. The observation of reversible or irreversible damage wrought by the surgical procedure indirectly supports the plea of Kistner[8] to relegate the surgical approach to a minor position in patients with Stein-Leventhal syndrome. It stresses the importance of meticulous technique in the performance of conservative ovarian surgery during the reproductive age. Patients with pelvic adhesions, impairing ovarian and tubal function, should be offered the benefit of reconstructive surgery.

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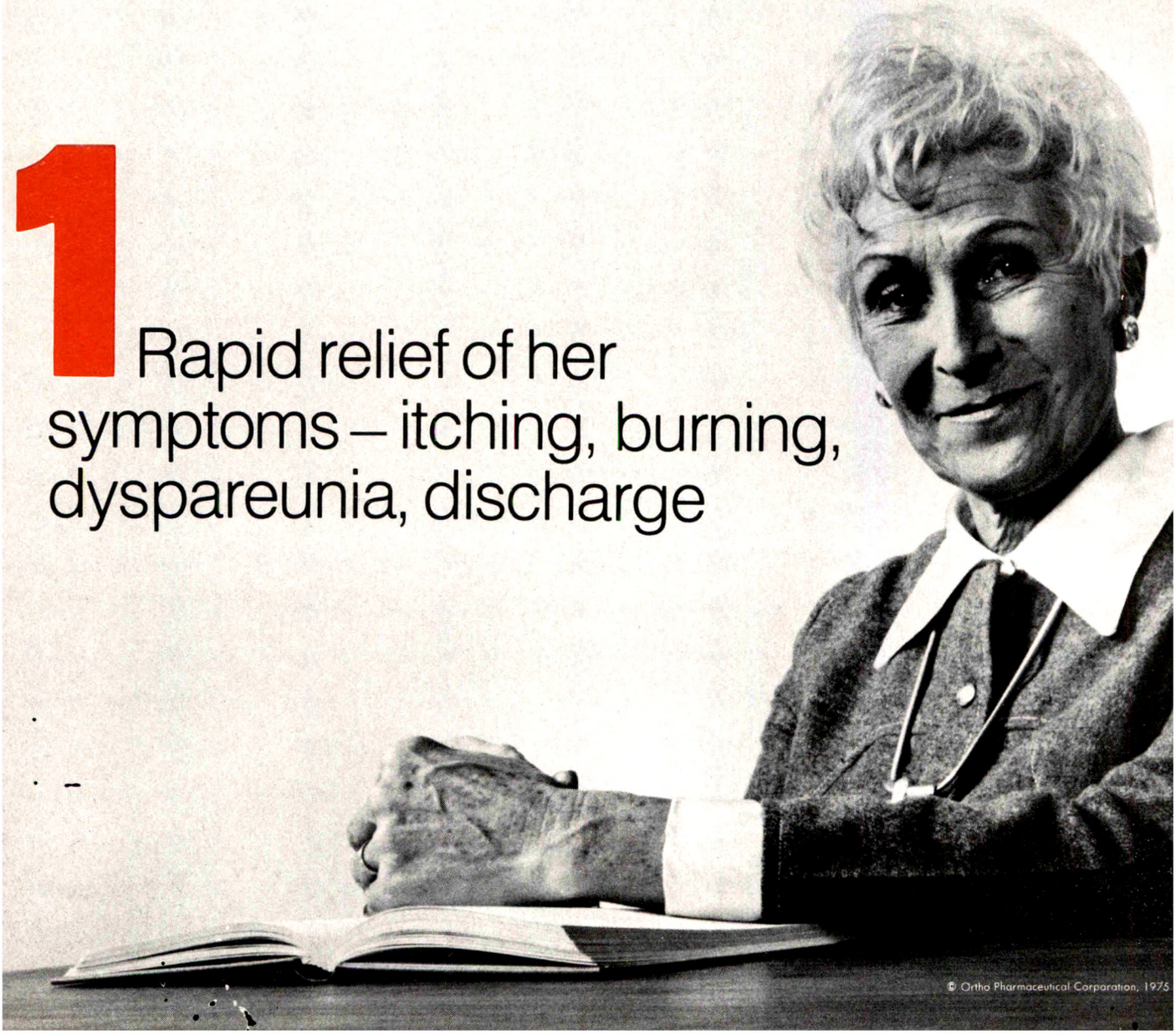
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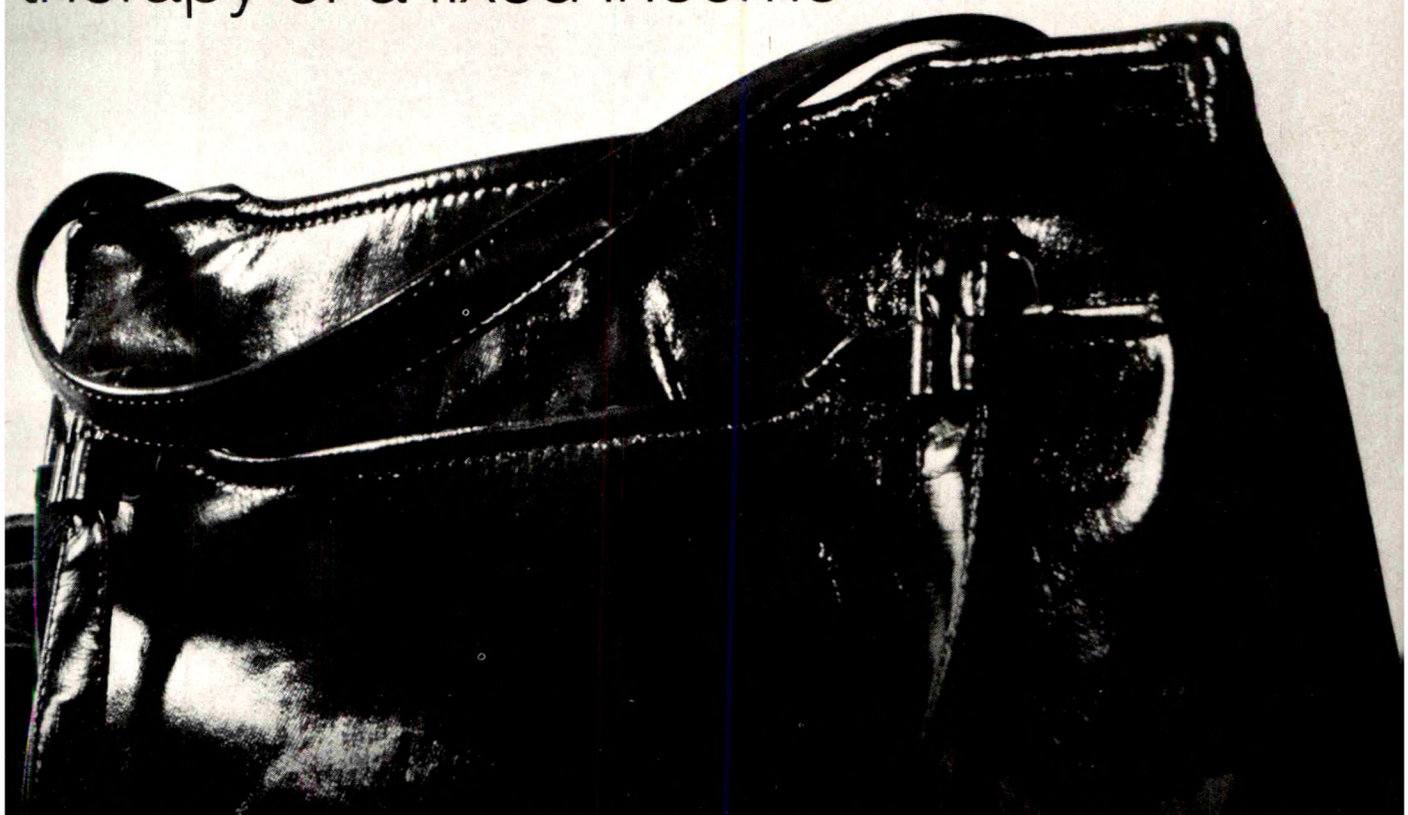
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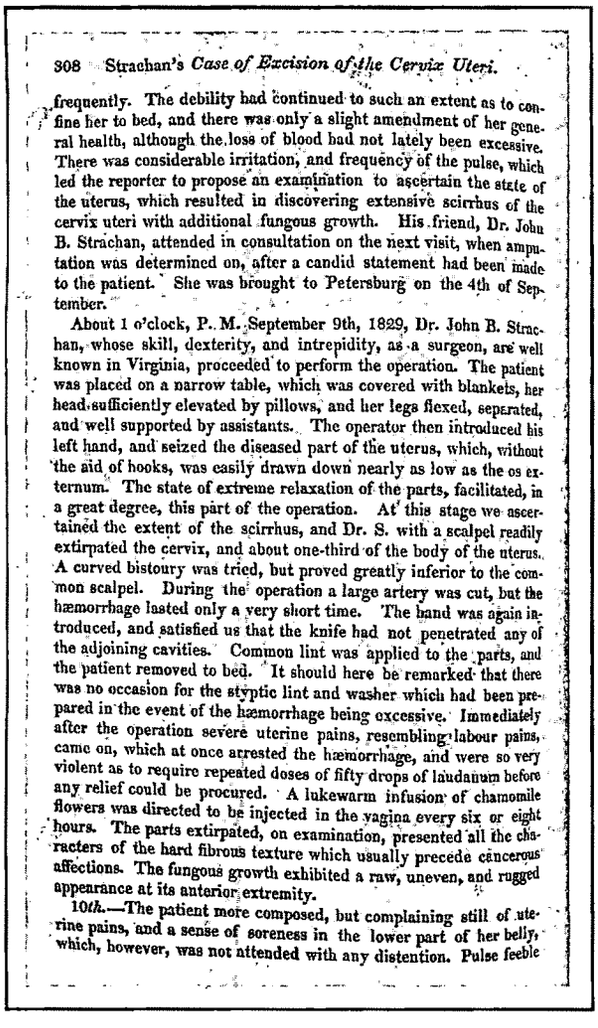
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Case of successful excision of the cervix uteri in a scirrhous state

JOHN B. STRACHAN
American Journal of the Medical Sciences, vol. 5, pp. 307-309, 1829



308 Strachan's *Case of Excision of the Cervix Uteri.*

frequently. The debility had continued to such an extent as to confine her to bed, and there was only a slight amendment of her general health, although the loss of blood had not lately been excessive. There was considerable irritation, and frequency of the pulse, which led the reporter to propose an examination to ascertain the state of the uterus, which resulted in discovering extensive scirrhus of the cervix uteri with additional fungous growth. His friend, Dr. John B. Strachan, attended in consultation on the next visit, when amputation was determined on, after a candid statement had been made to the patient. She was brought to Petersburg on the 4th of September.

About 1 o'clock, P. M. September 9th, 1829, Dr. John B. Strachan, whose skill, dexterity, and intrepidity, as a surgeon, are well known in Virginia, proceeded to perform the operation. The patient was placed on a narrow table, which was covered with blankets, her head sufficiently elevated by pillows, and her legs flexed, separated, and well supported by assistants. The operator then introduced his left hand, and seized the diseased part of the uterus, which, without the aid of hooks, was easily drawn down nearly as low as the os externum. The state of extreme relaxation of the parts, facilitated, in a great degree, this part of the operation. At this stage we ascertained the extent of the scirrhus, and Dr. S. with a scalpel readily extirpated the cervix, and about one-third of the body of the uterus. A curved bistoury was tried, but proved greatly inferior to the common scalpel. During the operation a large artery was cut, but the hæmorrhage lasted only a very short time. The hand was again introduced, and satisfied us that the knife had not penetrated any of the adjoining cavities. Common lint was applied to the parts, and the patient removed to bed. It should here be remarked that there was no occasion for the styptic lint and washer which had been prepared in the event of the hæmorrhage being excessive. Immediately after the operation severe uterine pains, resembling labour pains, came on, which at once arrested the hæmorrhage, and were so very violent as to require repeated doses of fifty drops of laudanum before any relief could be procured. A lukewarm infusion of chamomile flowers was directed to be injected in the vagina every six or eight hours. The parts extirpated, on examination, presented all the characters of the hard fibrous texture which usually precede cancerous affections. The fungous growth exhibited a raw, uneven, and rugged appearance at its anterior extremity.

10*th*.—The patient more composed, but complaining still of uterine pains, and a sense of soreness in the lower part of her belly, which, however, was not attended with any distention. Pulse feeble

Prior to the advent of cellular pathology, the classification of cervical malignancy was hopelessly confused. Treatment of this condition consisted of purging, venesection, leeches applied to the cervix, and other equally ineffective remedies. Amputation of the cervix gained some popularity as it occasionally met with success. Strachan, of Virginia, first successfully amputated the cervix in America. The patient, age 33, had been treated intermittently for 9 months for vaginal bleeding. Following operation (see figure) the patient developed severe pelvic pain and apparently infection, attended by temperature and rapid pulse. However, within five weeks, the wound was healed and the patient returned home.

CURRENT DEVELOPMENTS

Vaginal agenesis

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Vaginal agenesis is not rare. Many ingenious procedures have been devised for its correction; some are simple, and some are complicated. Not enough emphasis has been placed on the emotional aspects when patients with this problem are treated. Proper timing of the operation and selection of the best procedure are discussed. We feel the procedure of choice is the simplest, least dangerous one which will give good results in the individual patient. Rather than using the same routine procedure in each patient, we fitted the treatment to each patient. Our experience, particularly with the simple vulvovaginoplasty technique, is given. Postoperative support and encouragement are needed to have a successful outcome.

VAGINAL AGENESIS was first described in 1572 by Realdus Columbus.[1, 2] It is said to occur once in 4,000 to 5,000 women.[1, 3, 4]

Through the years, numerous ingenious techniques have been devised to treat this anomaly. Perhaps not enough emphasis has been given to the patient and her emotional status.

Material and methods

This report covers our experience, attitudes, and management techniques as they have evolved with the last 50 patients referred to this service for the creation of a neovagina. The distribution of patients (Table I) is as would be expected in a pediatric and adolescent gynecology service, i.e., there is a larger proportion of patients with intersex problems than seen in the usual gynecology service.

Some of these patients at this writing have had definitive treatment; others are waiting until we feel the proper time has arrived. While these youngsters are growing up, they are not simply waiting in a vacuum. We see the children and their families regularly for counseling and emotional support.

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The patient: emotional aspects and time of operation

Most important in the preoperative evaluation is assessment of the patient's emotional status and, indeed, that of the family also. Most patients can be properly handled by the gynecologist without need for psychiatric referral.

We stress the positive with emphasis on the normal—normal breasts, normal hormone production, normal pituitary, normal ovaries, normal sex hair, etc. We point out that the patient *can* marry and *can* have normal sexual relations with normal orgasm. She *can* have children. She will adopt her children, as will one of six married couples.

These patients, while not needing psychiatric care, do need emotional support, guidance, assurance, and tender loving care from the gynecologist. This, we feel very strongly, is part and parcel of the management of

Table I. Vaginal agenesis: distribution of 50 patients

Classification	*No. of patients*
Genetically normal female, vaginal agenesis only	13
Genetically normal female, vaginal agenesis with other congenital anomalies	17
Intersex patients, 4 with testicular feminization syndrome and 10 male hermaphrodites	20
Total	50

the patient with vaginal agenesis. To be simply a technician is not enough. One must be a keen technician and also a sensitive physician to help the patient over the emotional hurdles. It is also necessary that other members of the family be informed of the situation and what to expect.

It has been our observation that the patient with vaginal agenesis does not feel that she is a whole woman. She will not compete for dates with her peers for the eligible men in the community. She feels that she cannot develop an honest close relationship with a man.

Since she lacks the organ (vagina) which will permit her to be a complete woman, she feels she is deceiving the men she dates. Therefore, she avoids the conflict by not dating. In discussing this with behavioral scientists, we have learned that they have made similar observations.

We perform the operation when the patient feels that she wants the neovagina and when we are convinced that she will cooperate with the use of dilators postoperatively. If she is not ready to cooperate, the operation is doomed to failure because of atresia of the new vaginal canal. Operation should not be performed before the patient reaches full body size because the artificial vagina will not grow with the patient.

Some advocate that a definitive operation should be performed shortly before the patient is to be married. We prefer not to postpone the operation until shortly before marriage because at times the results are poor. If infection takes place or if constriction occurs, it may be necessary to postpone the wedding. This is an emotional catastrophe for the couple and a social catastrophe for the families.

In these 50 patients, there were one failure and one poor result because of inaccurate evaluation of the emotional status of the patient preoperatively. One patient had the McIndoe operation performed by a competent gynecologist in another city. We saw her one year later with no evident vaginal canal and much scar tissue. She had simply not cooperated postoperatively with the use of dilators because she "didn't feel like it." In her case, we performed a second operation with a large bowel transplant which will not obliterate if the patient does not cooperate.

Another of our patients who was 17 years old had a less than satisfactory result following the McIndoe operation because she would cooperate on an "off and on" basis. The vagina is not of adequate depth, and there is much scar tissue in the vault.

Which patients need vaginoplasty?

Patients with congenital vaginal agenesis will fall into one of the following categories: (1) the subject who is genetically female and normal except for vaginal agenesis (with or without a functioning uterus); (2) the subject who is genetically female with vaginal agenesis (with or without a functioning uterus) and other anomalies in the skeletal system, urinary tract, or bowel, etc.; (3) the intersex patient—the true hermaphrodite or the male hermaphrodite of either the masculinizing or feminizing type.

Other patients needing vaginal construction are those not having a functioning vagina secondary to cancer therapy—either surgical or radiation.

In addition, a new group of patients, the transsexuals, in some cases need vaginoplasty.

Diagnosis of vaginal agenesis

In the absence of functioning endometrial tissue, the most common complaint is primary amenorrhea, and most of our patients present in their teens with this complaint. Those seen later will also complain of inability to have satisfactory coitus.

If the patient has a functioning uterus, she will probably present at menarche with an acute picture of hematometra with pain and mass in the lower abdomen. If the upper portion of the vagina is present, she will also have hematocolpos.

The diagnosis of vaginal agenesis is extremely simple and can be made by examination of the patient. The usual findings are those of a normal vulva with normal labia majora, labia minora, urethra, and clitoris. There may be a shallow dimple measuring 1 or 2 cm. but no additional vaginal canal. This dimple represents the attempt of the urogenital sinus to form the lower vagina. Agenesis of the upper four fifths of the vagina results from failure of normal Müllerian duct development.

After examination of the vulva, rectal examination will usually confirm that there is no palpable uterus or cervix in most cases.

The classic vaginal agenesis patient with the so-called Rokitansky-Küster-Hauser Syndrome has no functioning uterus. Instead there are bilateral noncanalized muscular (myometrial) buds, normal tubes, normal round ligaments, and normal ovaries.

The rest of the physical examination usually shows normal breast development and normal sex hair, i.e., normal secondary sexual characteristics.

There are a number of conditions which may appear to be congenital absence of the vagina. These include adhesions of the labia minora,[5] the imperforate hymen, the microperforate hymen,[6] and labioscrotal fusion as seen in the adrenogenital syndrome and other forms of intersexuality.

If the hymen is imperforate, it may be noted to bulge shortly after birth from mucocolpos[7, 8] or at puberty from hematocolpos.

In the delivery room, if there is any question, it is easy to ascertain the presence of a vagina by inserting a lubricated catheter into the vaginal canal. This is not done routinely. Another simple way of confirming the presence of a vagina—at least the lower portion of the vagina—is to grasp the labia on both sides between the thumb and index finger and to gently pull the labia away from the patient toward the examiner while simultaneously spreading them laterally.[9]

This will expose and stretch the hymenal opening, and through it one can see the lower vaginal canal and mucosa. To be positive that there are an upper vagina and cervix, one must resort to vaginoscopy. This need be done only in a few selected cases.

The extent of the preoperative evaluation

After a thorough history and physical examination, two procedures are indicated. One is an intravenous pyelogram. A high percentage of patients with vaginal agenesis also have urinary tract anomalies such as absence of one kidney, horseshoe kidney, pelvic kidney, or duplicate collecting systems. Our studies have shown that 50 per cent of our patients with vaginal agenesis have a urinary tract anomaly.

Although not routinely done by us, spinal x-rays may reveal vertebral anomalies.[10–12]

A buccal smear or perferably chromosome studies are indicated to prove a normal female genetic status, that is sex chromatin positive and 46,XX chromosome pattern.[13–15]

Exploratory laparotomy or laparoscopy is not necessary in a genetically normal female subject if there is nothing to suggest a functioning uterus.

Before a definitive operation, the gynecologist must assure himself that the patient will cooperate with the use of vaginal dilators and molds postoperatively, or the result may be failure. This is the single most important aspect of the preoperative evaluation of the patient with vaginal agenesis.

Techniques currently in vogue

A number of ingenious procedures have been devised to treat this problem. Each has its advantages and disadvantages. One must evaluate each patient individually and use the simplest technique which will give good results in the particular patient involved. In other words, the procedure is chosen that best suits the individual patient.

The Frank nonoperative technique (Fig. 1). This depends on the use of dilators to make a channel by pressure between the bladder and rectum. In some highly motivated patients, this works well.[16] Indeed, cases are reported of formation of a neovagina by coitus alone.[17] Our experience has been that success is more likely in those patients who have a vaginal dimple of 3 or 4 cm. Without an appreciable dimple, the results with the Frank nonoperative technique are not good in our patients.

The Wharton technique (Fig. 2). A new vaginal space is dissected between the bladder and the rectum. A mold is placed into this space, and epithelialization should take place. This epithelialization takes a long period of time, and frequently there is considerable scarring high in the vault.

The McIndoe procedure[18] (Fig. 2). This is begun as in the Wharton operation. A split-thickness skin graft is taken from the buttock or thigh, placed over a stent, and inserted into the neovaginal canal to serve as a lining. This operation takes considerable time to perform and a long period of hospitalization. There is considerable risk of injury to bladder and rectum. Sometimes there is poor take of the graft, especially if infection takes place. Dilators must be used faithfully until regular coitus occurs, or the neovagina will constrict and become obliterated.

Procedures with the use of the bowel.[19-21] Other procedures involve transplantation of segments of small or large bowel. We feel that these bowel transplants are contraindicated except where other procedures have failed. It does not seem appropriate to increase the risk with bowel surgery until other simple methods have failed.

Procedure with the use of peritoneum. A technique has been described in which a neovagina is created and then lined with peritoneum.[22] With simpler vulvovaginoplasty procedures now available, it appears an unnecessary risk to enter the peritoneal cavity.

Transplant of vagina.[23] This has been reported from mother to daughter in Greece. It seems unneces-

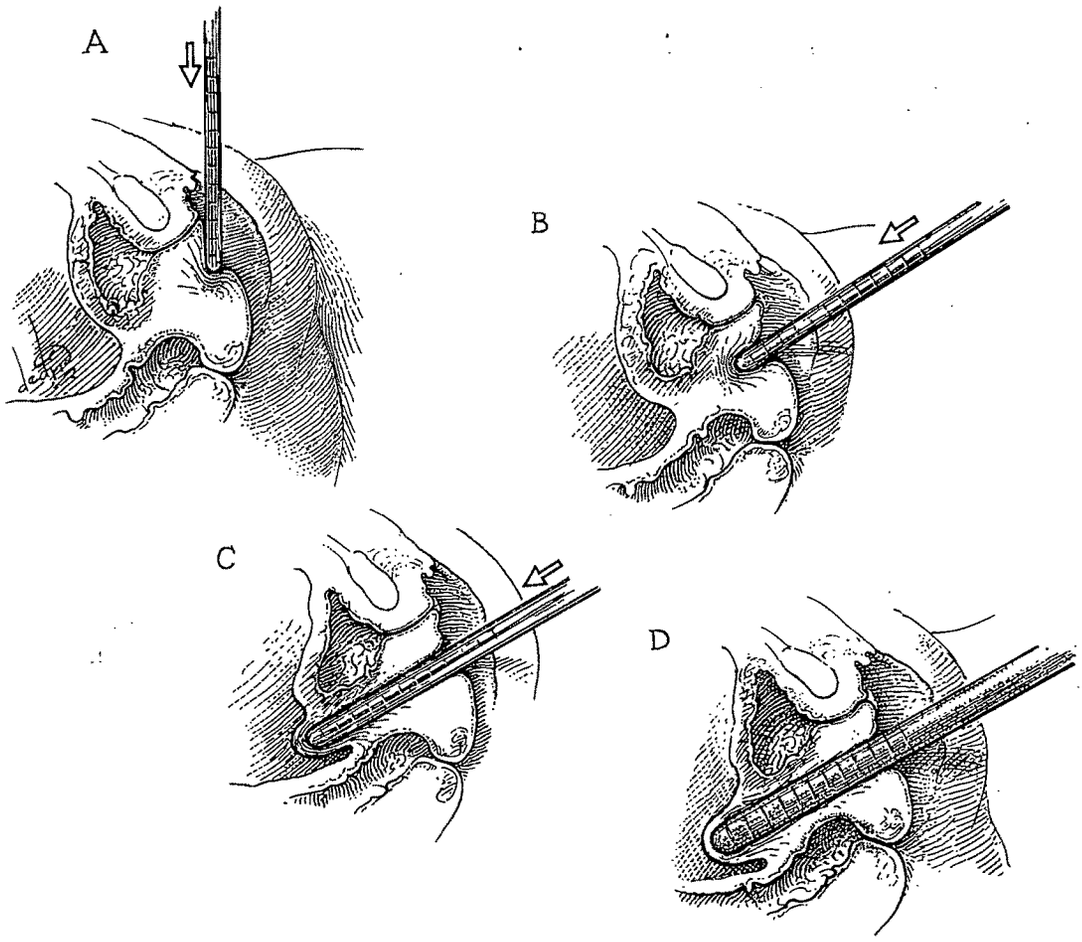


Fig. 1. Frank nonoperative technique of creating an artificial vagina. Dilators of increasing caliber are used to create an artificial channel by means of pressure. Note that in *A* the smallest dilator is directed posteriorly in order to avoid the urethra. After a short channel has been created, the dilators are then directed cephalad *(B, C,* and *D)* with longer and larger dilators. (Reprinted from the author's Chapter 39, Volume II, *Gynecology and Obstetrics,* by permission of the Medical Department, Harper & Row, Publishers, Inc., Hagerstown, Maryland.)

sary to place the patient at risk from immunosuppressive drugs when there are simpler and less risky alternative procedures available for vaginal reconstruction.

The Williams[24] vulvovaginoplasty technique. This involves use of the tissues of the labia. We first learned of this procedure in 1967 from Professor C. J. Dewhurst of London. Professor Williams[24] had first reported this in 1964 in patients where vaginectomy had been performed for malignancy. Our modification of this technique is described in Figs. 3 to 7.

The Buffalo modification of the Williams vulvovaginoplasty.

Our modification[25, 26] includes the following:

1. The incision begins at the level of the urethra or below. This permits free egress of urine and avoids the "cesspool" effect of stagnant urine trapped in the neovagina as might occur in the original technique.

2. The visual appearance of vulva is more "normal." The new "perineum" does not cover the urethra, and the normal anatomy of the upper labia minora and clitoris is preserved.

3. The "touch picture" of the vulva is more "normal" for the reasons already given.

4. With the more median placement of our incision, the hair-bearing skin of the labia majora is not part of the wall of the neovagina.

Why does this operation work?

In our experience, the Frank nonoperative technique is successful only where the patient has some vaginal canal (3 to 4 cm.) to begin with. The neovagina

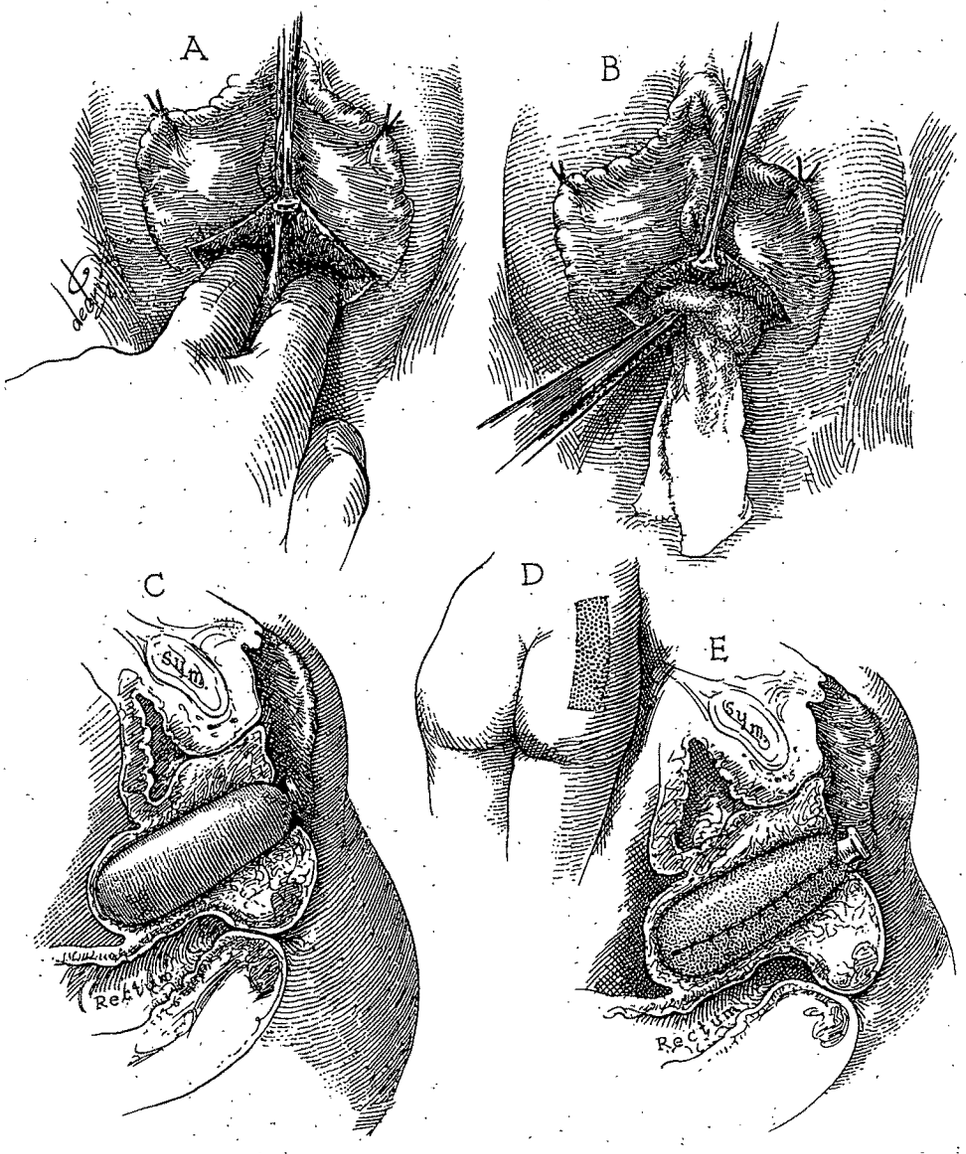


Fig. 2. Wharton technique and McIndoe technique of vaginoplasty. In both of these operative techniques, an artificial channel is developed between the bladder and rectum. *A,* Dissection is more easily carried out on both sides of the midline in loose adventitia-type tissue by blunt finger dissection. The adherent fibrous midline tissue, which is probably noncanalized Müllerian duct remnants, is then cut off with the scissors. *B,* Repeated packing of the canal with wet gauze aids in enlarging the cavity and in controlling fine capillary bleeding. *C,* Insertion of a vaginal mold completes the Wharton operation. The vaginal mold keeps the canal patent while spontaneous epithelialization occurs slowly. *D* and *E,* Completion of the McIndoe operation. *D,* The donor site for a split-skin graft. It is taken from the buttock so that it may be covered in a bathing suit and also because the patient cannot easily see it to be constantly reminded of her defect. *E,* A mold covered with a split-skin graft placed in the new vaginal canal for immediate epithelialization completes the McIndoe vaginoplasty. (Reprinted from the author's Chapter 39, Volume II, *Gynecology and Obstetrics,* by permission of the Medical Department, Harper & Row, Publishers, Inc., Hagerstown, Maryland.)

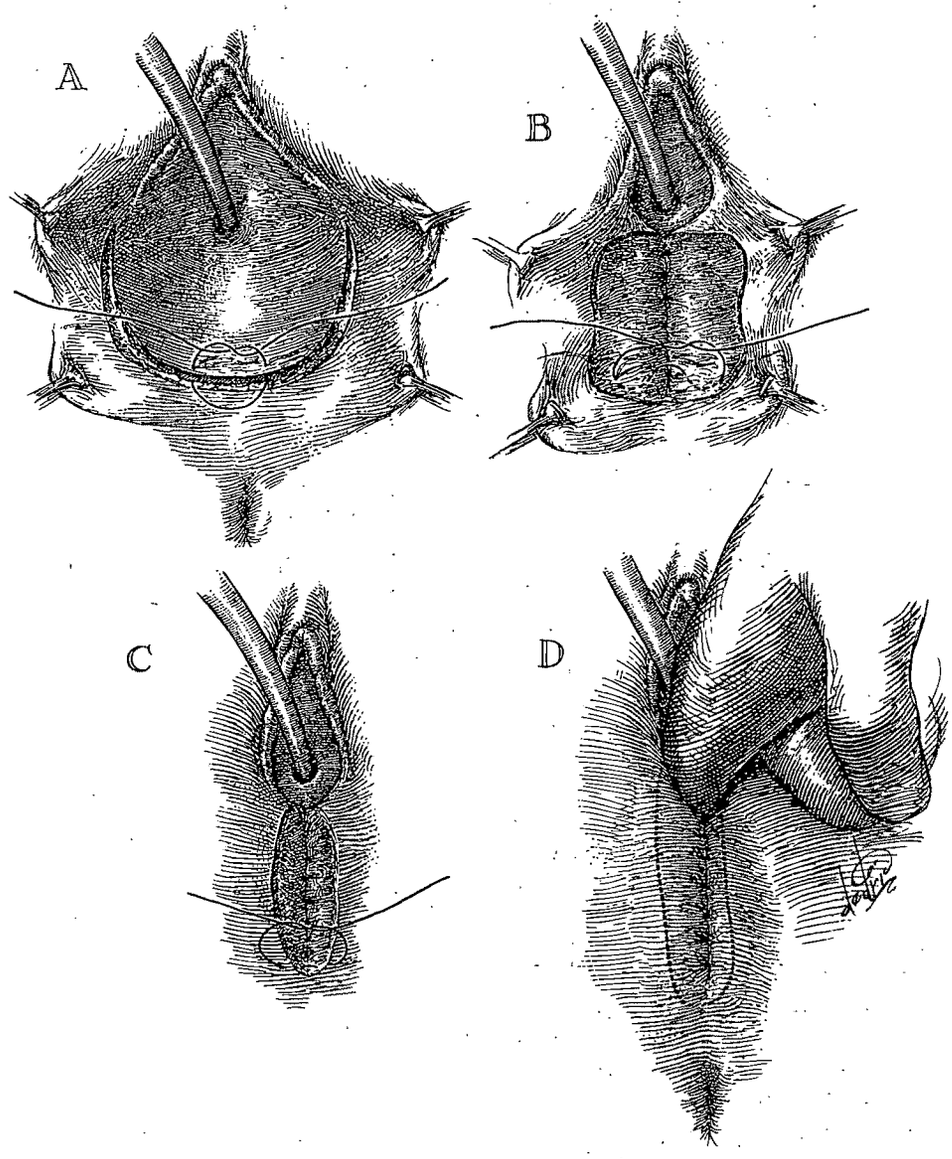


Fig. 3. *A,* The vulvar tissues are put under tension by four properly placed Allis clamps. A U-shaped incision in the skin of the medial aspects of the labia majora begins at the level of the urethra or lower, about 4 cm. lateral to the urethra. The closure of the first layer of skin begins posteriorly and proceeds anteriorly. Interrupted sutures of 3-0 chromic catgut on an atraumatic needle are tied so that the knots are placed in the new vaginal cavity rather than buried in the tissue. *B,* Closure of the second skin layer brings the subcutaneous tissue together in the midline with interrupted 3-0 catgut sutures. *C,* Closure of the third layer approximates skin and subcutaneous tissue with interrupted 3-0 or 4-0 chromic catgut sutures. *D,* The index finger is easily admitted into the neovagina. Axis of the neovagina is perpendicular to the floor immediately after operation. (Reprinted from the author's Chapter 39, Volume II, *Gynecology and Obstetrics,* by permission of the Medical Department, Harper & Row, Publishers, Inc., Hagerstown, Maryland.)

obtained by our method is smaller in caliber and depth than one obtained with the Williams method but has less distortion of "perineal tissues." The patient can easily lengthen and dilate the neovagina and change its axis by using test tubes (Figs. 5, 6, and 7) and later by active coitus. In this operation we construct a small neovagina "outward." Although this resembles a "kangaroo pouch," it does give the patient something to work with in order to develop the neovagina "inward." Normal depth, caliber, and axis (Fig. 6) have been easily achieved with dilators (Fig. 7) first and then coitus by 100 per cent of our patients.

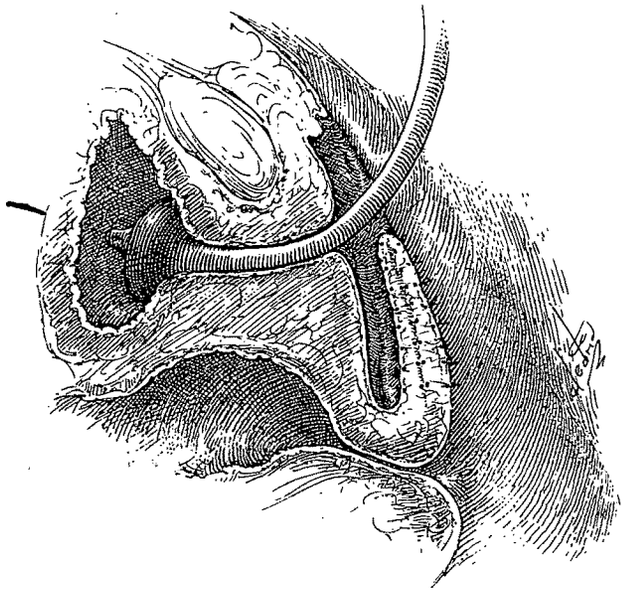


Fig. 4. Sagittal view shows direction of neovagina immediately after operation. Patients have not had any difficulty voiding. (Reprinted from the author's Chapter 39, Volume II, *Gynecology and Obstetrics,* by permission of the Medical Department, Harper & Row, Publishers, Inc., Hagerstown, Maryland.)

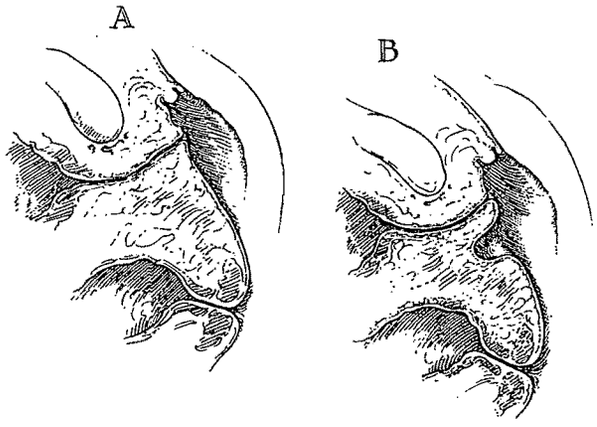


Fig. 5, *A* and *B*. *A,* Patient with no vaginal dimple. Frank nonoperative technique usually fails. *B,* Patient with vaginal "dimple" of at least 3 or 4 cm. is more likely to have good results with Frank nonoperative technique.

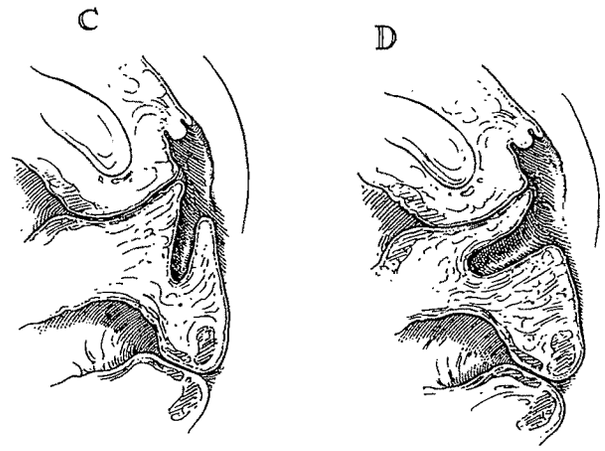


Fig. 5, *C* and *D*. *C,* Neovagina constructed "outward" with vulvovaginoplasty technique. This gives the patient something to work with. *D,* Neovagina of normal depth, caliber, and axis after use of dilators and then coitus.

Advantages of vulvovaginoplasty

The advantages of this vulvovaginoplasty technique for creating a neovagina are numerous:

1. The time of operation is reduced to minutes rather than hours—30 minutes average time for vulvovaginoplasty compared to 2½ hours for the McIndoe procedure.

2. The time of hospitalization is drastically reduced from weeks to days.

3. The risk of injuries to the urethra, bladder, or rectum is almost nil as compared to that with the other procedures.

4. The blood loss is minimal—only a few milliliters.

5. Eliminating all evidence and reminders of the anomaly is important in these young patients. With our procedure, there no visible abdominal scar (which occurs with bowel procedures) and no scarred skin donor site (which occurs with the McIndoe procedure).

6. There is no need for additional anesthesia to remove or change the vaginal mold as in the other operations.

7. It is possible to use the neovagina within a few weeks for coitus rather than having to wait months.

8. If the patient does not use the dilators, the neovagina will not constrict down as it does in the skin graft technique. It will remain patent because it is lined with normal mucosa. Dilatation at a future date will reconstitute its desired size.

9. Another important advantage of this procedure is that the patient does not have an artificial vagina. She has a neovagina which is made from her own vulvar tissues and not from a graft taken from the buttock. This psychological boost to the patient's ego and self-image is an important aspect.

Postoperative management with vulvovaginoplasty

An indwelling silicone rubber Foley catheter is inserted at operation and is removed in four or five

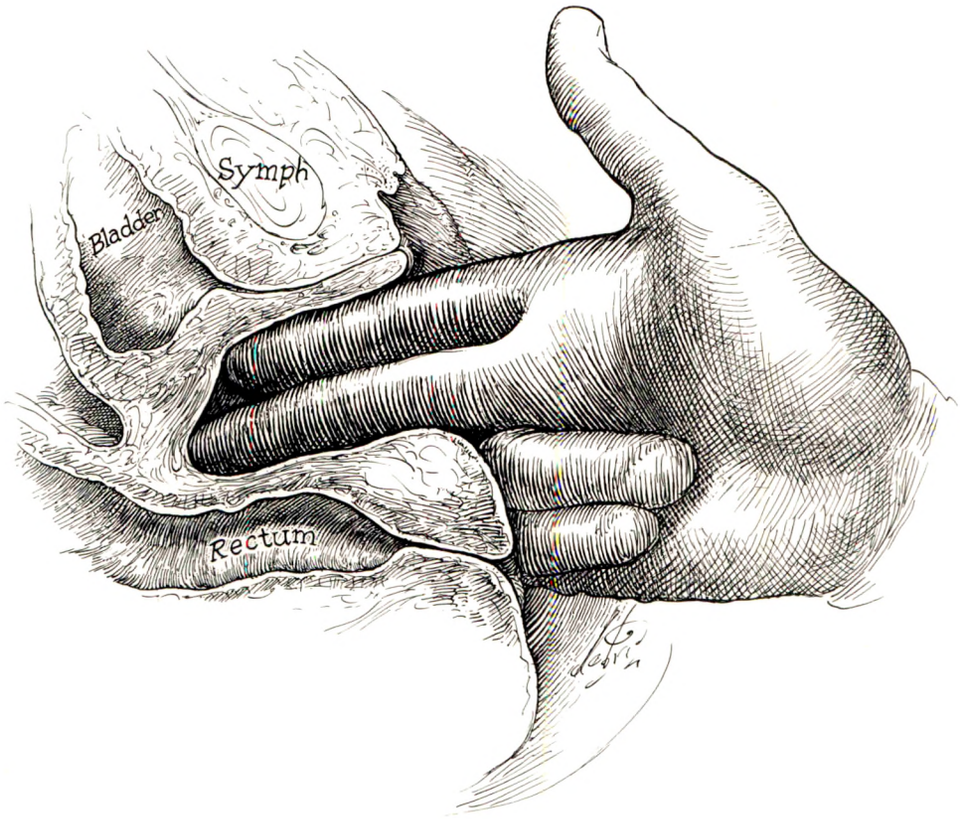


Fig. 6. Sagittal view after use of graded dilators and after sexual intercourse. The axis, depth, and caliber of the neovagina are found to be normal on digital vaginal examination.

days. Prophylactic postoperative antibiotics are given. Perineal care consists of soap and water washing plus use of a dry heat lamp. On the fourth or fifth day, we instruct the patient in the use of graded dilators, after which she is discharged. An important aspect of this procedure is close supervision of the patient to be sure she is using the dilators correctly and regularly.

Within a few weeks, the neovagina shows a normal axis and is of adequate depth and caliber (Fig. 6).

Results with the various techniques

Table II shows the techniques used in managing our last 50 patients with vaginal agenesis. Our results are listed below.

Frank nonoperative technique. Of the nine patients in whom the Frank nonoperative technique was used, all nine had adequate follow-up. Eight showed a good anatomic result, and in one the vaginal canal was not deep enough. Seven of the patients had coitus, which six stated was satisfactory and one stated was unsatisfactory. Three of the patients at this point have not had coitus.

Table II. Vaginal agenesis: Management of 50 patients

Type of procedure	*Operation performed*	*Operation contemplated at proper age*
Frank nonoperative	9	0
Wharton	3	0
McIndoe	7	10
Bowel transplant	2	0
Vulvovaginoplasty	13	2
To be determined	–	4
Total	34	16

Wharton technique. Of the three patients in whom this technique was used, two had adequate follow-up. One showed a good anatomic result, and one showed a poor anatomic result. Both of the patients who were followed had coitus, and both stated it was satisfactory.

McIndoe technique. All seven patients on whom the McIndoe operation was performed had adequate follow-up. The anatomic results were judged to be good in six of the patients and not satisfactory in one

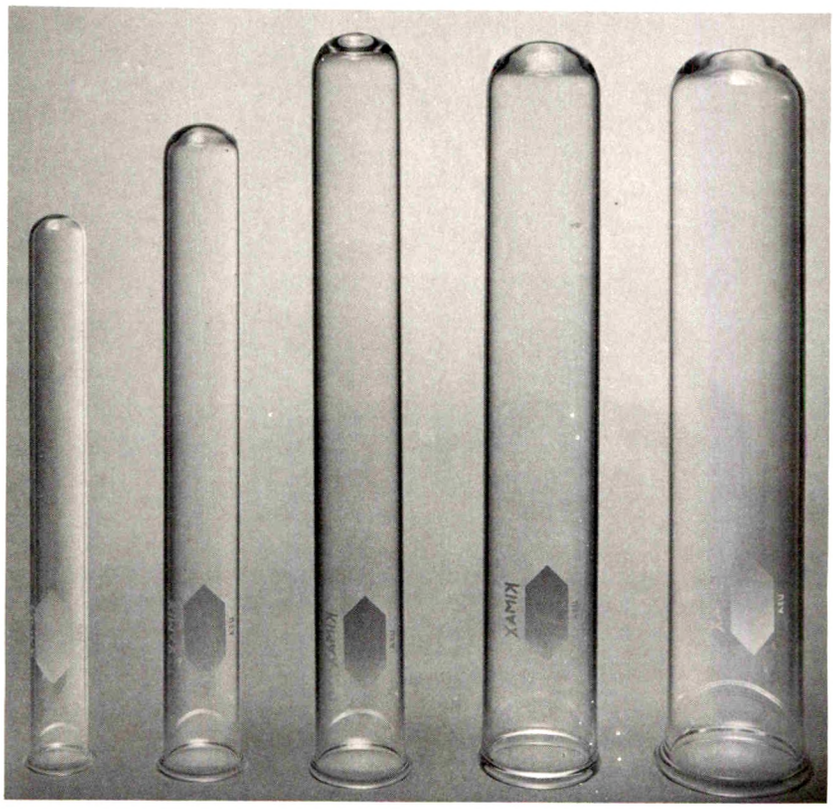


Fig. 7. Graded test tubes (outside diameter 1.8 to 3.7 cm.) used postoperatively as dilators by patient.

patient. Six of these patients reported having had coitus, and it was satisfactory in all six. One of the patients to this date has not had coitus.

Bowel transplant technique. The bowel transplant technique was used in two patients, both of whom had adequate follow-up. The anatomic result was good in both patients. Both patients have experienced coitus, which was described as satisfactory in one and not satisfactory in the other who complained of dyspareunia. This was corrected by the use of dilators.

Vulvovaginoplasty technique. Of the 13 patients in whom our modified vulvovaginoplasty technique was carried out, 12 had adequate follow-up. In 11 of these patients, the anatomic result was judged good on pelvic examination. In one patient the anatomic result was good insofar as depth and caliber of vagina. However, there was a slight breakdown in the lower portion of the sling which resulted from a local infection. Although we judged this a poor anatomic result, this patient has married and is having satisfactory coitus. Eight of the patients with this vulvovaginoplasty technique have experienced coitus, and all eight described this as satisfactory. Four of the patients with follow-up have not had coitus at the time of this writing.

The postoperative psychological status was good in all of the patients treated except for the two described previously.

Comment

An important aspect of management of the patient needing construction of a neovagina is recognition of the emotional problems involved. The timing of the operation is dependent on the patient's reaching full body size and on her willingness to cooperate postoperatively to prevent atresia of the neovagina.

At the present time, our choice of procedure depends on the anatomic findings. If the patient has an adequate "vaginal dimple" of 3 to 4 cm., the Frank nonoperative technique is tried first and generally succeeds. If it does not succeed, we then choose an operative method.

If the vulva appears normal with "good" labia, the vulvovaginoplasty as modified by us is our first choice. In the past four years, in genetically female subjects with normal labia, we have used this procedure exclusively.

If the vulvar anatomy is such that the labial structure is not present, as in some patients with intersex problems, we will choose the McIndoe operation with the use of a split-skin graft.

If any of these methods fail, we will then resort to a bowel transplant procedure. In other words, we use the simplest procedures first and reserve the more complicated and hazardous methods for when the other methods fail.

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Roster of American obstetrical and gynecological societies*

(Appears annually in the January 1 issue)

American Gynecological Society. (1876) *President,* Gordon W. Douglas. *Secretary,* J. George Moore, Dept. Ob-Gyn, UCLA School of Medicine, Los Angeles, Calif. 90024. Meetings, restricted.

The American Association of Obstetricians and Gynecologists. (1888) *President,* Keith Russell. *Secretary,* George W. Mitchell, Jr., 171 Harrison Ave., Boston, Mass. 02111. Meeting (Mid-year), April 15-17, 1976. Meeting (Annual), Sept. 9-11, 1976.

The Central Association of Obstetricians and Gynecologists. (1929) *President,* Raymond H. Kaufman. *Secretary,* David G. Anderson, Women's Hospital, University of Michigan Medical Center, Ann Arbor, Mich. 48104. Meetings, Oct. 14-16, 1976.

The South Atlantic Association of Obstetricians and Gynecologists. (1938) *President,* O. Hunter Jones. *Secretary,* William H. Kirkley, 2000 S. Andrews Ave., Ft. Lauderdale, Fla. 33316. Meetings, Jan. 24-29, 1976.

Pacific Coast Obstetrical and Gynecological Society. (1931) *President,* Carl Goetsch. *Secretary,* Leon J. Shulman, 2200 Santa Monica Blvd., Santa Monica, Calif. 90404. Meetings, Nov. 7-13, 1976.

Akron Obstetrical and Gynecological Society. (1934)

Atlanta Obstetrical and Gynecological Society. (1953) *President,* Thomas A. Harris. *Secretary,* Hugo S. Moreno, 2739 Felton Dr., East Point, Ga. 30344. Meetings, Jan. 6, March 5, and May 7, 1976.

Bronx Gynecological and Obstetrical Society. (1924)

Brooklyn Gynecological Society, Inc. (1890) *President,* Aldo C. Scorza. *Secretary,* Norman Ames Posner, 4802- 10th Ave., Brooklyn, N. Y. 11219. Meeting,

Buffalo Gynecologic and Obstetric Society. (1945) *President,* David H. Nichols, *Secretary,* M. M. Hreshchyshyn, 100 High St., Buffalo, N. Y. 14203. Meetings, second Tuesday, Sept.-April, 1976.

*Changes, omissions, and corrections must be received by the publisher two months in advance (by November 1) for the Roster. Please address the Journal Editing Department, The C. V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63141. The number after the Society's name is the year of founding. For further information, address the respective secretaries.

The American College of Obstetricians and Gynecologists. (1951) *President,* Roy T. Parker. *Secretary,* Martin L. Stone, New York Medical College, 1249 Fifth Ave., New York, N. Y. 10029. Meetings, May 10-13, 1976.

Section on Obstetrics and Gynecology, American Medical Association. (1859) *President,* John R. McCain. *Secretary,* Van R. Jenkins, Larkin Professional Building, 2505 Larkin Road, Lexington, Ky. 40503. Meeting, annual.

Society of Obstetricians and Gynaecologists of Canada. (1945) *President,* Lise Fortier. *Secretary,* John C. G. Whetham, 367 Lytton Blvd., Toronto, Ontario M5N 1R9. Meeting, June 8-12, 1976.

American Board of Obstetrics and Gynecology, Inc. (1930) *President,* J. George Moore. *Secretary,* Clyde L. Randall, 200 West Cold Spring Lane, Baltimore, Md. 21210. Meetings, June 22-23 and Nov. 5-12, 1976.

Chicago Gynecological Society. (1878) *President,* Lester D. Odell. *Secretary,* Robert E. Lane, 333 East Superior St., Chicago, Ill. 60611. Meetings, third Friday, except Dec., July, Aug., and Sept.

Cincinnati Obstetrical and Gynecological Society. (1876) *President,* Paul Haas. *Secretary,* Harold Johnstone, Bethesda Hospital, Oak & Reading Rd., Cincinnati, Ohio 45206. Meetings, third Thursday, each month.

Cleveland Society of Obstetricians & Gynecologists. (1947)

The Colorado Gynecological and Obstetrical Society.

Columbus Obstetric-Gynecologic Society. (1944)

Connecticut Society of American Board Obstetricians and Gynecologists, Inc. (1952) *President,* Peter Goodhue. *Secretary,* Philip C. Dennen, 134 Grandview Ave., Suite 203, Waterbury, Conn. 06708. Meetings, May and Dec.

Dallas-Fort Worth Obstetric and Gynecologic Society. (1933) *President,* Robert B. Brunken. *Secretary,* E. E. Harrison, 202-B, Lake Highlands Village, Dallas, Texas 75218. Meetings, third Tuesday, April and Oct.

Dayton Obstetrical and Gynecological Society. (1932)

Georgia State Obstetrical and Gynecological Society. (1951) *President,* John S. Inman. *Secretary,* Armand Hendee, 69 Butler St., S.E., Atlanta, Ga. 30703. Meetings, March and Dec., 1976.

Honolulu Obstetrical and Gynecological Society. (1947) *President,* Gail Li. *Secretary,* Theodore Tseu, 1523 Kalakaua Ave., Honolulu, Hawaii 96814. Meetings, variable date.

Idaho Obstetrical and Gynecological Society. (1955) *President,* Max D. Gudmundsen. *Secretary,* Dean T. Watkins, 148 E. Jefferson, Boise, Idaho 83702. Meeting, spring 1976.

Indiana Obstetrical and Gynecological Society. (1947) *President,* Charles R. Echt. *Secretary,* David E. Copher, 3266 N. Meridian #404, Indianapolis, Ind. 46208. Meetings, three times yearly.

Iowa Obstetrical and Gynecological Society. (1946) *President,* John G. Napier. *Secretary,* Robert M. Kretzschmar, University Hospitals, Iowa City, Iowa Meetings, Dec. 10-11, 1975.

Jackson Gynecic Society. President, Thomas E. Holden. Secretary-Treasurer, Fred Ingram, Department of Obstetrics and Gynecology, University of Mississippi Medical Center, 2500 N. State St., Jackson, Mississippi 39216. Meetings, fourth Monday of each month, excluding June, Aug., and Dec.

Kansas City Gynecological Society. (1932) *President,* William T. Griffin. *Secretary,* Thomas L. Stribling, 3036 Gillham Road, Kansas City, Mo. 64108. Meetings, Jan. 15, March 18, and May 20, 1976.

The Kentucky Obstetrical and Gynecological Society. (1948) *President,* Mervel V. Hanes. *Secretary-Treasurer,* Hugh P. Adkins, 303 Medical Towers North, Louisville, Ky. 40402. Meetings, spring and fall.

Louisville Obstetrical and Gynecological Society *President,* J. Patrick Sutherland. *Secretary,* Charles R. Oberst, 215 Medical Towers North, Louisville, Ky. 40202.

Los Angeles Obstetrical and Gynecological Society, Inc. (1914) *President,* Lester T. Hilbard. *Secretary,* James C. Caillouette, 5410 Wilshire Blvd., Suite 600, Los Angeles, Calif. 90036. Meetings, second Tuesday, Jan. 13, March 9, and May 18, 1976.

Memphis and Shelby County Obstetrical and Gynecologic Society. *President,* Martha Loving. *Secretary,* Jesse C. Woodall, Jr., 220 S. Claybrook, Suite 401, Memphis, Tenn. 38104. Meetings, second Tuesday of each month, excluding June, July, and Aug.

Miami Obstetrical and Gynecological Society. (1946) *President,* Howard A. Novell. *Secretary,* Arthur I. Rudolph, 7400 N. Kendall Dr., Suite 410, Miami, Fla. 33056. Meetings, second Thursday, Nov., Jan., March, and May.

Michigan Society of Obstetricans and Gynecologists. (1924) *President,* Hermann A. Ziel, Jr. *Secretary,* Robert G. Walkowiak, 28 W. Adams, Detroit, Mich. 48226. Meetings, Jan. 3, Feb. 12, March 9, April 4, and May 15, 1976.

Milwaukee Gynecological Society. (1951) *President,* David V. Foley. *Executive Secretary,* Robert Herzog, 2825 N. Mayfair Rd., Milwaukee, Wis. 53222. Meetings, Feb. 9, March 8, April 12, Oct. 5, and Nov. 2, 1976.

Mississippi Obstetrical and Gynecological Society. (1947) *President,* Calvin T. Hull. *Secretary,* Lewis D. Lipscomb, 500 G. E. Woodrow Wilson, Jackson, Miss. 39216. Meeting, May 5, 1976.

Montana Obstetrical and Gynecological Society. (1948) *President,* Ed Schumaker. *Secretary,* Gordon K. Phillips, 1220 Central Ave., Great Falls, Mont. 59401. Meetings, unscheduled.

Nassau Obstetrical and Gynecological Society. (1944) *President,* James N. Nicklas. *Secretary,* J. Conrad Greenwald, 179 Community Dr., Great Neck, N. Y. 11020. Meetings, Mar. 1 and April 26, 1976.

Nebraska State Obstetric and Gynecological Society. (1955) *President,* Marvin Dietrich. *Secretary,* Dennis D. Beavers, 8552 Cass St., Omaha, Neb. 68114. Meetings, Dec. 4-6.

New Jersey Obstetrical and Gynecological Society. (1947) *President,* Robert Haines. *Secretary,* James Thompson, 2424 Morris Ave., Union, N. J. 07083. Meeting, annual.

The New Orleans Gynecological and Obstetrical Society. (1947)

The New York Obstetrical Society. (1863) *President,* James Nelson. *Secreatry,* E. Thomas Steadman, 449 E. 68 St., New York, N. Y. 10021. Meetings, second Tuesday, Oct. through May, 1976.

North Dakota Society of Obstetrics and Gynecology. (1938) *President,* Joseph Adducci. *Secretary,* Robert P. Bushell, 737 Broadway, Fargo, N. D. 58102. Meeting, Sept. 10-11, 1976.

Northern California Obstetrics and Gynecologic Society. (1953) *President,* John Evrigenis. *Secretary,* John J. Sullivan, Campus Commons Medical Plaza, 1 Scripps Drive. Suite 305, Sacramento, Calif. 95825. Meetings, third Wednesday.

Obstetrical and Gynecological Assembly of Southern California. (1945) *General Chairman,* James E. Davis. *Secretary,* Bruce B. Rolf, 5410 Wilshire Blvd., Suite 600, Los Angeles, Calif. 90036. Meetings, Feb. 9-13, 1976.

Obstetrical Society of Boston. *President,* Charles L. Easterday. *Secretary,* Edward H. Copenhaver, 605 Commonwealth Avenue, Boston, Mass. 02215. Meetings, Jan. 19, Feb. 9, March 15, April 19, Oct. 18, and Nov. 15.

The Obstetrical Society of Philadelphia. (1868) *President,* John J. Mikuta. *Secretary,* Kaighn Smith, Suite 433, Lankenau Medical Building, Philadelphia, Pa. 19151. Meetings, first Thursday, each month, except Jan.

Oklahoma City Obstetrical and Gynecological Society. (1940)

Omaha Obstetrical-Gynecological Society. (1946) *President,* William Orr. *Secretary,* Joseph Scott, Dept. Ob-Gyn, University of Nebraska Medical Center, Omaha, Neb. 68132. Meetings, Jan., Mar., May, Sept., and Nov.

Orange County Obstetrical & Gynecological Society. (1958)

Pediatric Section of the County of Kings and the Academy of Medicine. *President,* Shirley M. Lippsett. *Secretary,* A. Zachary Apfel, 105-23 Ave., Brooklyn, N. Y. Meetings, Jan. 14, Feb. 18, and April 17, 1976.

Peninsula Gynecologic Society. (1954) *President,* Frederick R. Schlichting. *Secretary,* Arthur M. Brown, 596 Carroll St., Sunnyvale, Calif. 94086. Meetings, Feb., May, Sept., and Nov.

Philadelphia Obstetrical Society.

Phoenix Obstetrical and Gynecological Society. *President,* Norman Brown. *Secretary,* Franklin Loffer, 2021 N. Central Ave., Phoenix, Ariz. 85004. Meetings, Jan., March, May, Sept., and Nov.

Queens Gynecological Society. (1948) *President,* Patrick J. Concannon. *Secretary,* John S. Rienzo, c/o Queens Gynecological Society, 112-25 Queens Blvd., Forest Hills, Queens. Meetings, Feb. 11 and May 12, 1976.

Riverside-San Bernardino Obstetrical and Gynecological Society. (1957) *President,* William G. McCormick. *Secretary,* Jerome Niswonger, Loma Linda University Medical Center, Dept. Ob-Gyn, Room 3004, Loma Linda, Calif. 92354. Meetings, Jan. 21, Mar. 10, and May 19, 1976.

Rochester Academy of Medicine, Obstetrics and Gynecology Section. (1939) *President,* Henry Thiede. *Secretary,* Marvin Amstey, Highland Hospital, Rochester, N. Y. 14620. Meetings, quarterly.

St. Louis Gynecological Society. (1922) *President,* Denis Cavanagh. *Secretary,* Joe E. Belew, 3839 Lindell Blvd., St. Louis, Mo. 63108. Meetings, Feb. 12 and April 8, 1976.

San Antonio Obstetrical and Gynecological Society. (1963) *President,* Paul Weinberg. *Secretary,* Wendell Schorlemer, 318 Medical Professional Bldg., San Antonio, Texas 78212. Meetings, third Wednesday, Jan., March, May, July, Sept., and Nov.

San Diego Gynecological Society. (1937) *President,* Roger Miesfield. *Secretary,* Richard Philpott, 3240 Main St., Lemon Grove, Calif. 92045. Meetings, third Thursday, June, July, and Aug.

San Francisco Gynecological Society. (1929) *President,* Eugene C. Sandberg. *Secretary-Treasurer,* Robert W. Hoag, 2915 Telegraph Avenue, Berkeley, Calif. 94705. Meetings, second Tuesday, each month, except Dec. of each academic year.

Shufelt Society of Santa Clara County. (1947) *President* Harlan S. Parks, *Secretary,* Vincent F. Nola, 2081 Forest Ave., San Jose, Calif. 95128. Meetings, Jan. 15, Mar. 18, May 20, July 15, Sept. 16, and Nov. 18, 1976.

Society for Gynecological Investigation. *President,* Robert B. Jaffe. *Secretary,* Thomas H. Kirschbaum, Dept. of OB-GYN, Michigan State Univ., E. Lansing, Mich. 48824. Meeting, March 24-26, 1976.

South Dakota Society of Obstetrics and Gynecology. (1952) *President,* Milton G. Mutch, Jr. *Secretary,* Thomas L. Looby, 1200 S. Euclid, Sioux Falls, S. D. 57105. Meeting, annual.

Suffolk County Obstetrical and Gynecological Society. *President,* Jack Lipman. *Secretary,* Melvin Berlin, 111 Carleton Ave., Islip Terrace, N. Y. 11752. Meetings: second Tuesday, Sept., Nov., Feb., and May.

The Texas Association of Obstetricans and Gynecologists. (1931) *President,* William J. McGanity. *Secretary,* William H. Nash, Suite 304, 7711 Louis Pasteur Drive, San Antonio, Texas 78229. Meetings, Mar. 11-13, 1976.

Toledo Society of Obstetricians and Gynecologists. *President,* Carl Armstrong. *Secretary,* L. R. Kavanagh, 1614 S. Byrne Rd., Toledo, Ohio 43614. Meetings, Feb. and May, 1976.

Tri-Counties Obstetrical and Gynecological Society. (1959) *President,* Lowell McLellan. *Secretary,* Larry R. Moncur, 317 W. Pueblo St., Santa Barbara, Calif. 93105. Meetings, bi-monthly.

Utah Obstetrical and Gynecological Society. (1948) *President,* James Warenski. *Secretary,* Robert McMaster, 3905 Harrison Blvd., #308, Ogden, Utah 84403. Meetings, quarterly.

Virginia Obstetrical and Gynecological Society. (1936) *President,* Mason C. Andrews. *Secretary,* Lucien W. Roberts, P.O. Box 10, South Boston, Va. 24592. Meetings, spring and fall.

Washington Gynecological Society. (1933) *President,* Harzen S. Shea. *Secretary,* Charles E. Townsend, 4740 Bradley Blvd., Chevy Chase, Md. 20015. Meetings, Jan. 4, March 10, and April 14, 1976.

Westchester Obstetrical and Gynecological Society. (1937)

West Virginia Obstetrical and Gynecological Society. (1935) *President,* Robert P. Pulliam. *Secretary,* T. Keith Edwards, Bluefield Sanitarium, Bluefield, West Virginia 24701. Meeting, October 8-9, 1976.

Wisconsin Society of Obstetrics and Gynecology. *President,* William J. O'Leary, Jr. *Secretary,* C. Weir Horswill, 2630 Amhurst Rd., Middleton, Wisc. 53562. Meeting, early Sept., 1976 dates to be arranged.

ITEMS

Symposium on Diabetes and Other Endocrine Disorders During Pregnancy and in the Newborn Infant

A two-day Symposium on Diabetes and Other Endocrine Disorders During Pregnancy and in the Newborn Infant will be held at the New York Hilton Hotel in New York City, April 1-2, 1976. Sponsored by The National Foundation–March of Dimes, the symposium program will be presented by Cornell University Medical Center.

Dr. Maria I. New, Professor of Pediatrics, Cornell University Medical Center, and Dr. Robert H. Fiser, Jr., Professor and Chairman of the Department of Pediatrics, University of Arkansas Medical Sciences Campus, will serve as co-chairmen of the conference.

The purpose of this two-day symposium is to aid in understanding the perinatal physiology of diabetes and other hormonal disorders. Specific attention will be given to the effect on the fetus and the newborn infant of hormones administered to the mother during pregnancy. Guidelines for the therapeutic use of hormones and the management of the diabetic during pregnancy will be discussed.

The specific topics to be discussed are: (1) Diabetes and Carbohydrate Regulation of the Fetus and Newborn, (2) Gonadotrophins and Growth Factors, (3) Steroid Hormones During Pregnancy and in the Newborn, and (4) Thyroid and Parathyroid Hormones.

For further information, contact: The National Foundation–March of Dimes, 622 Third Ave., New York, New York 10017. Telephone: (212) 682-6430.

XIIIth Société Médicale Internationale d'Endoscopie et de Radiocinéma International Congress

The XIIIth Société Médicale Internationale d'Endoscopie et de Radiocinéma (S.M.I.E.R.) International Congress will take place in Lyons, France, May 6-8, 1976. Professor Pierre Mounier-Kuhn will preside. The Congress will be presented in liaison with the meeting of the French Language Broncho-Esophagology Society (President, Professor J. Gaillard). The theme will be, "The Contribution of Endoscopy, Radiology, Cinematography and Thermography Toward Early Diagnosis of Cancer." Official languages include French and English, with simultaneous translation. The Secretary-General–Treasurer is: Professeur Agrégé J. P. Haguenauer, O.R.L. Clinic, Hôpital de la Croix-Rousse, 69317 Lyon Cedex 1, France. The Agency is: LYCOTOUR, Maison de Lyon, Place Bellecour, B.P. 254 Lyon R.P., 69223 Lyon Cedex 1, France.

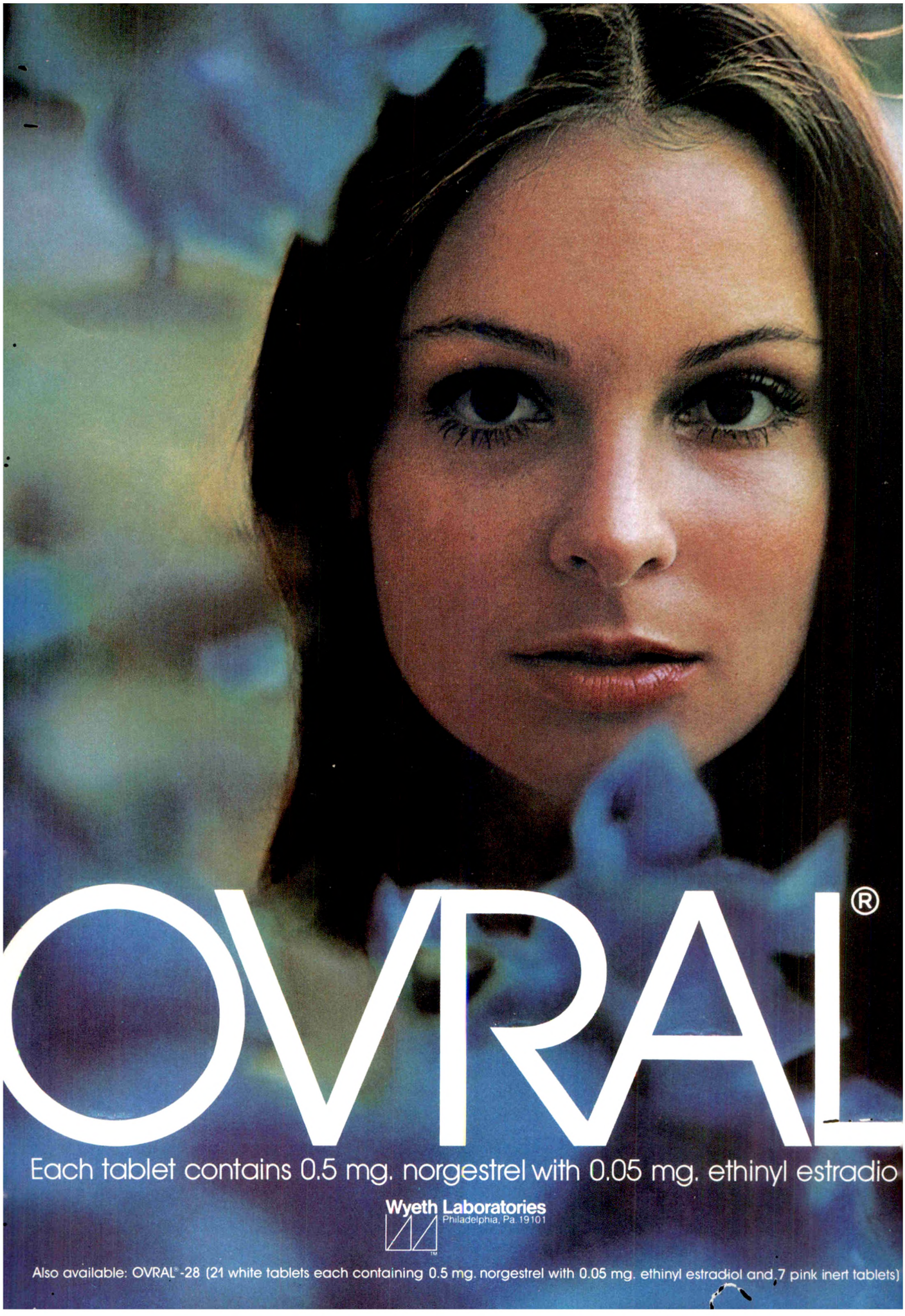
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Program directors are Philip J. DiSaia, M.D., and Roger K. Freeman, M.D. The guest faculty includes John McLean Morris, M.D., Felix N. Rutledge, M.D., and Robert E. Scully, M.D.

The program will be presented by the Women's Hospital Medical Center, Long Beach, California, January 16-18, 1976, in the Memorial Hospital Medical Center of Long Beach–University of California, Irvine Center for Health Education, 2801 Atlantic Ave., Long Beach, California. Registration fee is $200.00 ($100.00 for residents). This continuing medical education activity is acceptable for 15 hours of category 1 credit toward the Physicians' Recognition Award of the AMA.

Further information may be obtained from the Executive Secretary, Center for Health Education, 2801 Atlantic Ave., Long Beach, California 90801. Telephone: (213) 595-3823.


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Usha Mehra, M.D., Timothy O'Connor, M.B., B.Ch., Frank Ostapowicz, M.D., F.A.C.O.G., and Denis Cavanagh, M.D., F.A.C.O.G.
St. Louis, Missouri

Periovulatory changes in ovarian temperature in ewes
Henry J. Benoit, M.S.E.E., Rudi Borth, D.Sc., Allen R. Ellicott, Ph.D., and C. Allan Woolever, M.D.
Toronto, Ontario, Canada

Treatment of cervical carcinoma
Clayton T. Beecham, M.D., and David D. Beiler, M.D.
Danville, Pennsylvania

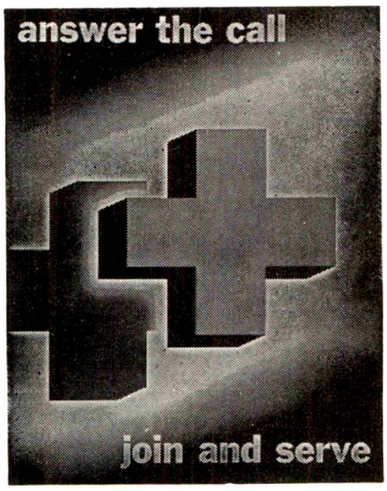
Inhibition of spontaneous contractility of the human and the rabbit Fallopian tube by potassium
A. Johns, S. Molnar, and D. M. Paton
Edmonton, Alberta, Canada

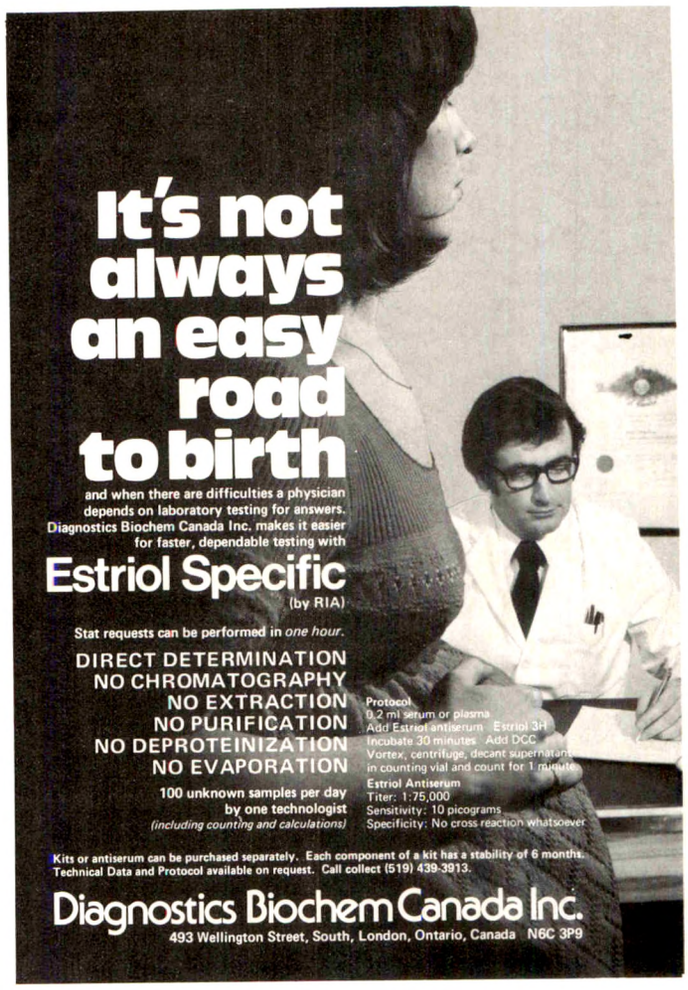
The effectiveness of two oral contraceptives in suppressing plasma androstenedione, testosterone, LH, and FSH, and in stimulating plasma testosterone-binding capacity in hirsute women
James R. Givens, Richard N. Andersen, Winfred L. Wiser, Edward S. Umstot, and Stewart A. Fish
Memphis, Tennessee

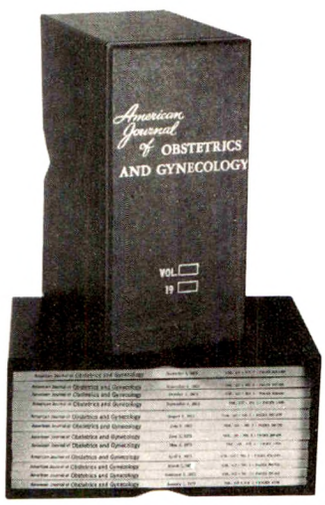
Effect of clomiphene citrate on serum prolactin in infertile women with ovarian dysfunction
Katsuyoshi Seki, M.D., Mitsunori Seki, M.D., Toshiko Okumura, M.D., and Ko-En Huang, M.D.
Chiba, Japan

Carcinoma in situ of the vagina following treatment for carcinoma of the cervix: A distinctive clinical entity
Glenn W. Geelhoed, M.D., Donald E. Henson, M.D., Peyton T. Taylor, M.D., and Alfred S. Ketcham, M.D.
Bethesda, Maryland

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For continued relief of postepisiotomy discomfort, send her home from the hospital with DERMOPLAST® Aerosol

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DERMOPLAST Aerosol Spray contains: Benzocaine, w/w 4.5%; benzethonium chloride, w/w 0.1%; menthol, w/w 0.5%; oxyquinoline benzoate, w/w 1.2%; and methylparaben, w/w 2.0%. Inactive ingredients, vegetable oils and propellant. Also contains 1.9% isopropyl alcohol.

For additional indications, full directions for use, and warnings, see label copy on DERMOPLAST Aerosol Spray can.



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January 15, 1976
volume 124, number 2



American Journal of OBSTETRICS AND GYNECOLOGY

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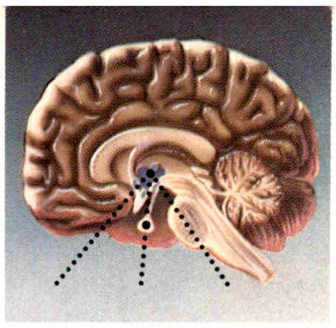
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When women outlive their ovaries...



Here, illustration shows artist's interpretation of cross section of brain symbolizing impulse transmissions from hypothalamic area to the pituitary and other neurohormonal target organs.

"There we were—my husband at the peak of his career—busy, successful...but no time for me. With that and all my other problems, I'd lie awake night after night, more depressed every day. This wasn't a 'change,' it was a catastrophe."



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Because some emotional symptoms may simply coincide with the menopause, it is essential to distinguish them from those symptoms which are related to estrogen deficiency–and to begin appropriate therapy.

It's axiomatic that the menopause carries its own burden of physical and emotional problems, some related to ovarian decline, some not.

The first step in identifying the source of emotional symptoms is to obtain a thorough history; emotional problems of recent origin are more likely to be related to estrogen deficiency than those of long standing.

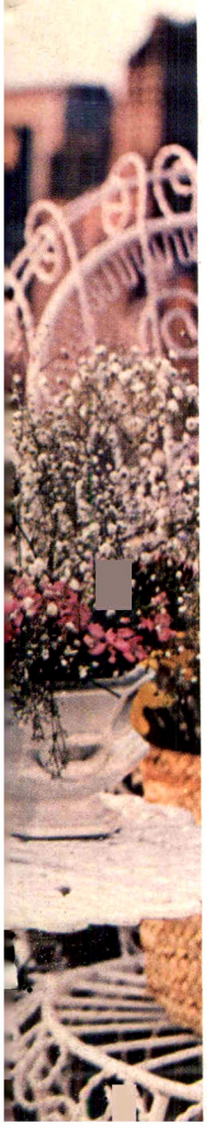
Then, to distinguish those symptoms that will respond to estrogen replacement therapy from those that may require other approaches to treatment, institute a *therapeutic trial* with PREMARIN.

In many cases, prolonged use of tranquilizers will not be necessary; patients taking PREMARIN alone often report relief of emotional symptoms when these are due to estrogen deficiency...and an improved sense of well-being.

Thus, the benefits of PREMARIN in such patients are threefold: it can help relieve symptoms related to estrogen deficiency; help treat the underlying cause; and finally, help identify symptoms unrelated to estrogen deficiency.

"I was always so irritable... snapping at the children... almost anything could annoy me. I knew I was doing it, but I just couldn't seem to stop myself."





For the emotional symptoms of the menopause related to estrogen deficiency

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(CONJUGATED ESTROGENS TABLETS, U.S.P.)



CONTAINS NATURAL ESTROGENS EXCLUSIVELY

See last page of advertisement for prescribing information.

BRIEF SUMMARY
(For full prescribing information, see package circular.)
PREMARIN® Brand of
CONJUGATED ESTROGENS TABLETS, U.S.P.

Actions: Estrogens are responsible for development and maintenance of the female reproductive system and secondary sex characteristics. They cause the growth and development of the vagina, uterus, and fallopian tubes, and enlargement of the breasts. Indirectly, they contribute to the shaping of the skeleton, maintenance of tone and elasticity of urogenital structures, changes in the epiphyses of the long bones that allow for pubertal growth spurt and termination, growth of axillary and pubic hair, and pigmentation of the nipples and genitals. Estrogens are also involved in psychologic and emotional aspects of feminine behavior. As estrogen levels increase during the menstrual cycle, there is a sense of vigor and well-being, and in the menopausal period estrogens aid in relieving nervous symptoms (*e.g.* anxiety, depression and irritability) due to estrogen deficiency. Decline of estrogenic activity at the end of the menstrual cycle can bring on menstruation, although the cessation of progesterone secretion is the most important factor in the mature ovulatory cycle. However, in the pre- or non-ovulatory cycle, estrogen is the primary determinant in the onset of menstruation. Estrogens do not induce ovulation.

Estrogens affect calcium and phosphorus metabolism, and are involved in maintenance of normal bone structure. In prolonged estrogen deficiency states, administration of estrogen acts to prevent associated bone degenerative changes.

Estrogens appear to be responsible for the relatively greater amount of alpha-lipoprotein and correspondingly lower amount of beta-lipoprotein found in premenopausal women as compared with men.

Estrogens affect the release of pituitary gonadotropins and may also deplete the gonadotropic content of the pituitary.

Indications: Based on a review of PREMARIN Tablets by the National Academy of Sciences—National Research Council and/or other information, FDA has classified the indications for use as follows:
Effective: As replacement therapy for naturally occurring or surgically induced estrogen deficiency states associated with: the climacteric, including the menopausal syndrome and postmenopause; senile vaginitis and kraurosis vulvae, with or without pruritus.
"Probably" effective: For estrogen deficiency-induced osteoporosis, and only when used in conjunction with other important therapeutic measures such as diet, calcium, physiotherapy, and good general health-promoting measures. Final classification of this indication requires further investigation.

Contraindications: Short acting estrogens are contraindicated in patients with (1) markedly impaired liver function; (2) known or suspected carcinoma of the breast, except those cases of progressing disease not amenable to surgery or irradiation occurring in women who are at least 5 years postmenopausal; (3) known or suspected estrogen-dependent neoplasia, such as carcinoma of the endometrium; (4) thromboembolic disorders, thrombophlebitis, cerebral embolism, or in patients with a past history of these conditions; (5) undiagnosed abnormal genital bleeding.

Warnings: Estrogen therapy should not be given to women with recurrent chronic mastitis or abnormal mammograms except, if in the opinion of the physician, it is warranted despite the possibility of aggravation of the mastitis or stimulation of undiagnosed estrogen-dependent neoplasia.

The physician should be alert to the earliest manifestations of thrombotic disorders (thrombophlebitis, retinal thrombosis, cerebral embolism and pulmonary embolism). If these occur or are suspected, estrogen therapy should be discontinued immediately.

Estrogens may be excreted in the mother's milk and an estrogenic effect upon the infant has been described. The long range effect on the nursing infant cannot be determined at this time.

Hypercalcemia may occur in as many as 15 percent of breast cancer patients with metastases, and this usually indicates progression of bone metastases. This occurrence depends neither on dose nor on immobilization. In the presence of progression of the cancer or hypercalcemia, estrogen administration should be stopped.

A statistically significant association has been reported between maternal ingestion of diethylstilbestrol during pregnancy and the occurrence of vaginal carcinoma in the offspring. This occurred with the use of diethylstilbestrol for the treatment of threatened abortion or high risk pregnancies. Whether or not such an association is applicable to all estrogens is not known at this time. In view of this finding, however, the use of any estrogen in pregnancy is not recommended.

Failure to control abnormal uterine bleeding or unexpected recurrence is an indication for curettage.

Precautions: As with all short acting estrogens, the following precautions should be observed:

A complete pretreatment physical examination should be performed with special reference to pelvic and breast examinations.

To avoid prolonged stimulation of the endometrium and breasts in climacteric or hypogonadal women, estrogens should be administered cyclically (3 week regimen with 1 week rest period—withdrawal bleeding may occur during rest period).

Because of individual variation in endogenous estrogen production, relative overdosage may occur which could cause undesirable effects such as abnormal or excessive uterine bleeding, mastodynia and edema.

Because of salt and water retention associated with estrogenic anabolic activity, estrogens should be used with caution in patients with epilepsy, migraine, asthma, cardiac, or renal disease.

If unexplained or excessive vaginal bleeding should occur, reexamination should be made for organic pathology.

Estrogen usage may cause preexisting fibromyomata to increase in size; therefore, patients should be examined at regular intervals while receiving estrogenic therapy.

The pathologist should be advised of estrogen therapy when relevant specimens are submitted.

Because of their effects on epiphyseal closure, estrogens should be used judiciously in young patients in whom bone growth is incomplete.

Prolonged high dosages of estrogens will inhibit anterior pituitary functions. This should be borne in mind when treating patients in whom fertility is desired.

The age of the patient constitutes no absolute limiting factor, although treatment with estrogens may mask the onset of the climacteric.

Certain liver and endocrine function tests may be affected by exogenous estrogen administration. If test results are abnormal in a patient taking estrogen, they should be repeated after estrogen has been withdrawn for one cycle.

Adverse Reactions: The following adverse reactions have been reported associated with short acting estrogen administration:
nausea, vomiting, anorexia
gastrointestinal symptoms such as abdominal cramps and bloating
breakthrough bleeding, spotting, unusually heavy withdrawal bleeding (See DOSAGE AND ADMINISTRATION)
breast tenderness and enlargement
reactivation of endometriosis
possible diminution of lactation when given immediately postpartum
loss of libido and gynecomastia in males
edema
aggravation of migraine headaches
change in body weight (increase, decrease)
headache
allergic rash
hepatic cutaneous porphyria becoming manifest

Dosage and Administration: PREMARIN should be administered cyclically (3 weeks of daily estrogen and 1 week off) for all indications except selected cases of carcinoma and prevention of postpartum breast engorgement.

Menopausal Syndrome—1.25 mg. daily, cyclically. Adjust dosage upward or downward according to severity of symptoms and response of the patient. For maintenance, adjust dosage to lowest level that will provide effective control.

If the patient has not menstruated within the last two months or more, cyclic administration is started arbitrarily. If the patient is menstruating, cyclic administration is started on day 5 of bleeding. If breakthrough bleeding (bleeding or spotting during estrogen therapy) occurs, increase estrogen dosage as needed to stop bleeding. In the following cycle, employ the dosage level used to stop breakthrough bleeding in the previous cycle. In subsequent cycles, the estrogen dosage is gradually reduced to the lowest level which will maintain the patient symptom-free.

Postmenopause—as a protective measure against estrogen deficiency-induced degenerative changes (*e.g.* osteoporosis, atrophic vaginitis, kraurosis vulvae)—0.3 mg. to 1.25 mg. daily and cyclically. Adjust dosage to lowest effective level.

Osteoporosis (to retard progression)—usual dosage 1.25 mg. daily and cyclically.

Senile Vaginitis, Kraurosis Vulvae with or without Pruritus—0.3 mg. to 1.25 mg. or more daily, depending upon the tissue response of the individual patient. Administer cyclically.

How Supplied: PREMARIN (Conjugated Estrogens Tablets, U.S.P.)
No. 865—Each *purple* tablet contains 2.5 mg., in bottles of 100 and 1,000.
No. 866—Each *yellow* tablet contains 1.25 mg., in bottles of 100 and 1,000. Also in unit dose package of 100.
No. 867—Each *red* tablet contains 0.625 mg., in bottles of 100 and 1,000. Also in unit dose package of 100.
No. 868—Each *green* tablet contains 0.3 mg., in bottles of 100 and 1,000.

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Caution: Folic acid may obscure pernicious anemia; the peripheral blood picture may revert to normal while neurological manifestations remain progressive.

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PD-JA-0807-3-P (4-75)

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The causes of vaginitis are multiple



You can depend on AVC... therapy that combats major vaginal pathogens and provides rapid symptomatic relief.*

***Indications:** Based on a review of AVC by the National Academy of Sciences–National Research Council and/or other information, FDA has classified the indications as follows:

"Probably" effective: For the relief of symptoms of vulvovaginitis where isolation of the specific organism responsible (usually *Trichomonas vaginalis, Candida albicans,* or *Hemophilus vaginalis)* is not possible.

NOTE: When the offending organism is known, treatment with a specific agent known to be active against that microorganism is preferred.

"Possibly" effective: For the treatment of trichomoniasis, vulvovaginal candidiasis, and vaginitis due to *Hemophilus vaginalis* or other susceptible bacteria.

Final classification of the less-than-effective indications requires further investigation.

Contraindications: Known sensitivity to sulfonamides.

Precautions/Adverse Reactions: The usual precautions for topical and systemic sulfonamides should be observed because of the possibility of absorption. Burning, increased local discomfort, skin rash or other manifestations of sulfonamide toxicity are reasons to discontinue treatment.

Dosage: 1 applicatorful (about 6 g.) or 1 suppository intravaginally once or twice daily.

Supplied: Cream–4 ounce tube with applicator.
Suppositories–Box of 16 with inserter.

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References: 1. Kunin CM: *Detection, Prevention and Management of Urinary Tract Infections.* Philadelphia, Lea & Febiger, 1972, pp 17, 21. **2.** Whalley PJ: Bacteriuria of pregnancy. *Am J Obstet Gynecol* 97:723-727, 1967. **3.** Czerwinski AW, et al: Studies in the detection of bacteriuria, in Greifer I (ed): *Bacteriuria and Urinary Tract Infections,* Proceedings of the International Symposium of the National Kidney Foundation, New York, June 12-13, 1973, pp 85-95.

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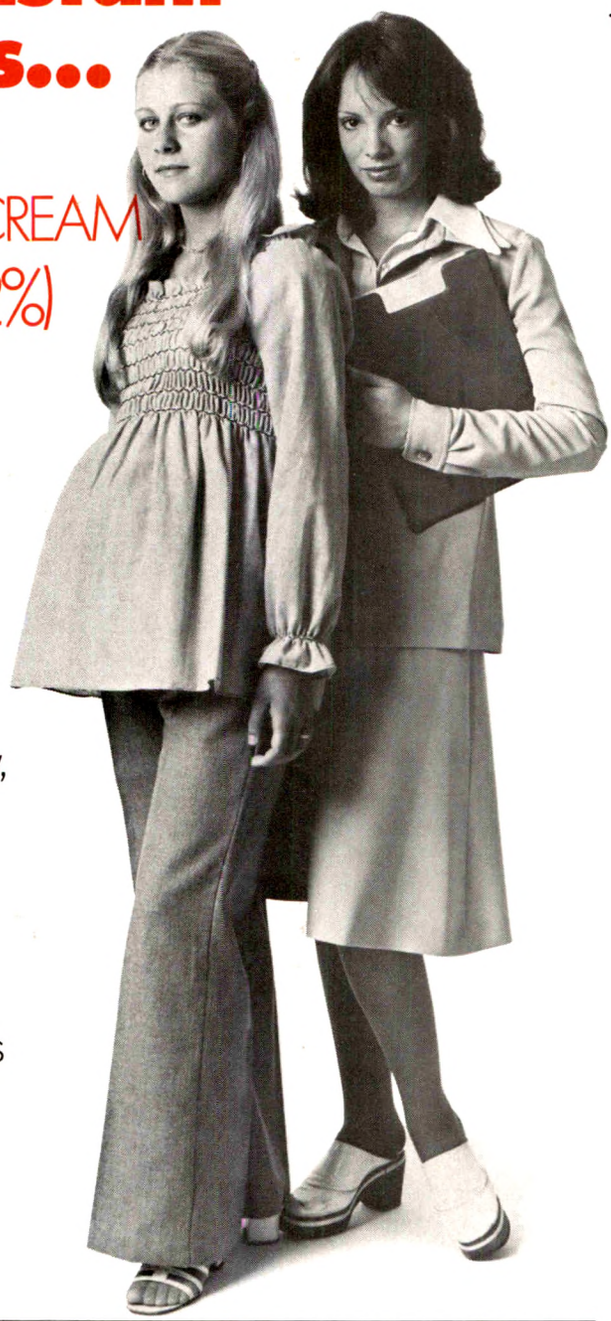
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Actions MONISTAT Cream exhibits fungicidal activity *in vitro* against species of the genus *Candida*. The pharmacologic mode of action is unknown.

Indications MONISTAT Cream is indicated for the local treatment of vulvovaginal candidiasis (moniliasis).

As MONISTAT Cream is effective only for candidal vulvovaginitis, the diagnosis should be confirmed by KOH smears and/or cultures. Other pathogens commonly associated with vulvovaginitis (*Trichomonas* and *Haemophilus vaginalis*) should be ruled out by appropriate laboratory methods.

MONISTAT is effective in both pregnant and non-pregnant women, as well as in women taking oral contraceptives. (See PRECAUTIONS.)

Contraindication Patients known to be hypersensitive to this drug.

Precautions *General:* Discontinue drug if sensitization or irritation is reported during use. *Laboratory Tests:* If there is a lack of response to MONISTAT, appropriate microbiological studies should be repeated to confirm the diagnosis and rule out other pathogens. *Pregnancy:* MONISTAT Cream was used in 209 pregnant patients. Follow-up reports now available in 117 of these patients reveal no adverse effects or complications attributable to MONISTAT therapy in infants born to these women. Since MONISTAT Cream is absorbed in small amounts from the human vagina, it should be used in the first trimester of pregnancy only when the physician considers it essential to the welfare of the patient.

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Dosage and Administration One applicatorful is administered intravaginally once daily at bedtime for 14 days. Course of therapy may be repeated after other pathogens have been ruled out by appropriate smears and cultures.

Supplied MONISTAT Cream is available in 3 oz. (85 g) tubes with Ortho* Measured Dose Applicator.

MONISTAT (Miconazole Nitrate 2%) is a development of Ortho Pharmaceutical Corporation and Janssen Pharmaceutica, Belgium.

*TRADEMARK

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Ortho Pharmaceutical Corporation Raritan, New Jersey 08869

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***INDICATIONS:** Replacement therapy for naturally occurring or surgically induced estrogen deficient states, including the menopause syndrome and post-menopausal conditions.*

***CONTRAINDICATIONS:** Women having familial or personal history of mammary or genital cancer.*

***PRECAUTIONS:** Estrogen should not be administered to women with recurrent mastitis or abnormal mammograms except in instances where the physician feels that treatment is warranted despite the possibility of aggravation of the mastitis or stimulation of undiagnosed estrogen dependent neoplasia.*

The physician should be alert to the earliest manifestation of thrombotic disorders. Should this problem occur, discontinue estrogen therapy immediately. The long range effect of estrogen on nursing infants has not been established.

Hypercalcemia may occur in as many as 15 percent of breast cancer patients with metastases, and this usually indicates progression of bone metastases. This occurrence depends neither on dose nor on immobilization. In the presence of progression of the cancer of hypercalcemia, estrogen administration should be discontinued. The use of any estrogen in pregnancy is not recommended.

A complete pretreatment physical examination should be performed with special reference to the pelvis and breast. To avoid continuous stimulation of the uterus and breast, cyclic therapy is recommended (3 weeks on medication and 1 week off). Withdrawal bleeding may occur during the one week off medication. If unexplained or excessive vaginal bleeding occurs, the physician should check for the organic pathology.

Pre-existing fibromyomata may increase in size while using estrogen. For this reason, patients should be examined at regular intervals. Treatment with estrogen may mask the onset of the climacteric. Certain liver and endocrine function tests may be affected by exogenous estrogen administration.

***DOSAGE:** Orally, for the relief of menopausal symptoms, HORMONIN NO. 1, 1 or 2 tablets daily according to severity of symptoms; HORMONIN NO. 2, 1 tablet daily. The initial dosage may be decreased to establish maintenance dosage. To avoid possible continuous stimulation of the breast and uterus, cyclic therapy may be employed. In severe cases, initial therapy may be supplemented with parenteral estrogens.*

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DIV. G. W. CARNRICK CO., 65 HORSE HILL ROAD, CEDAR KNOLLS, N.J. 07927

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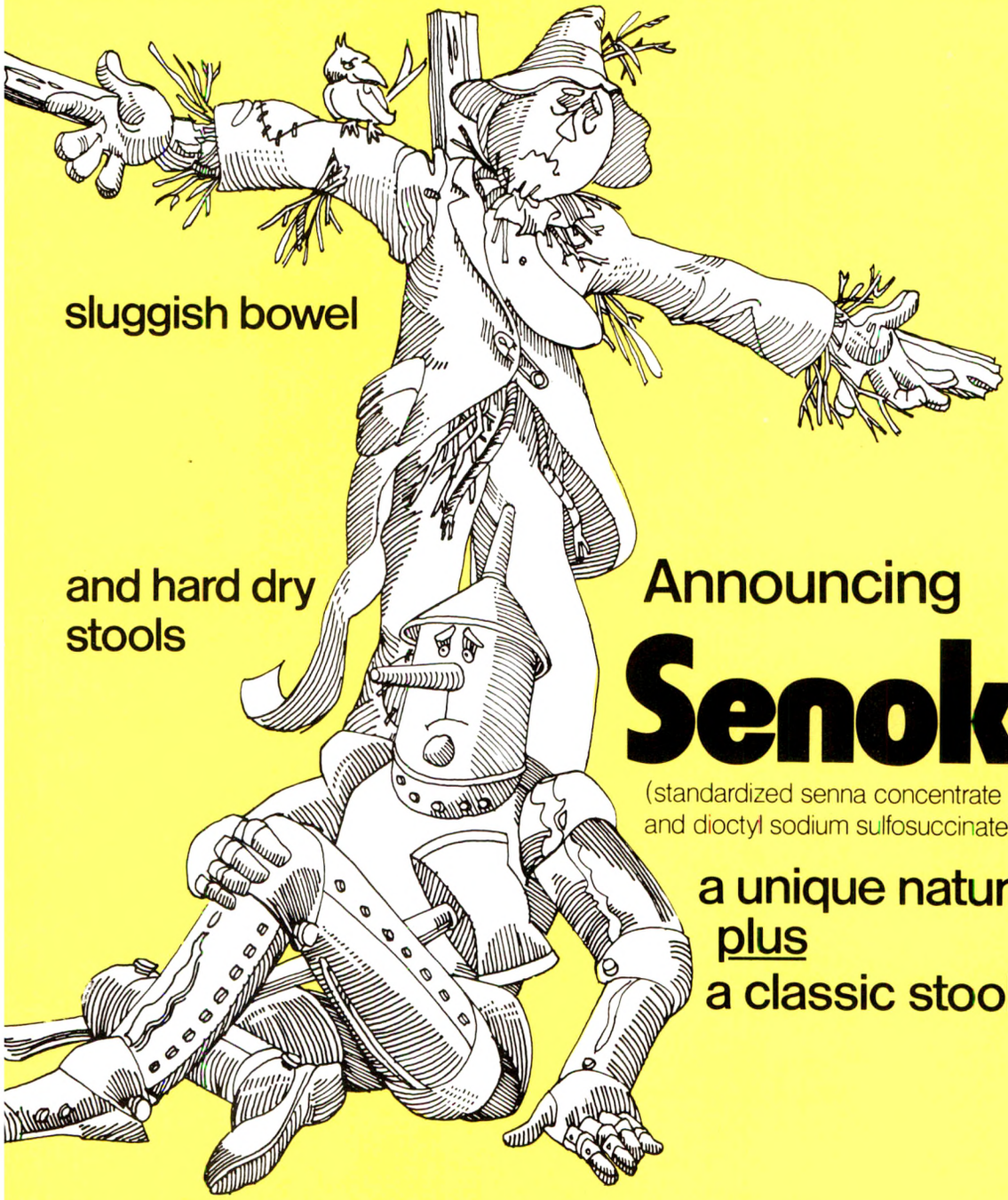
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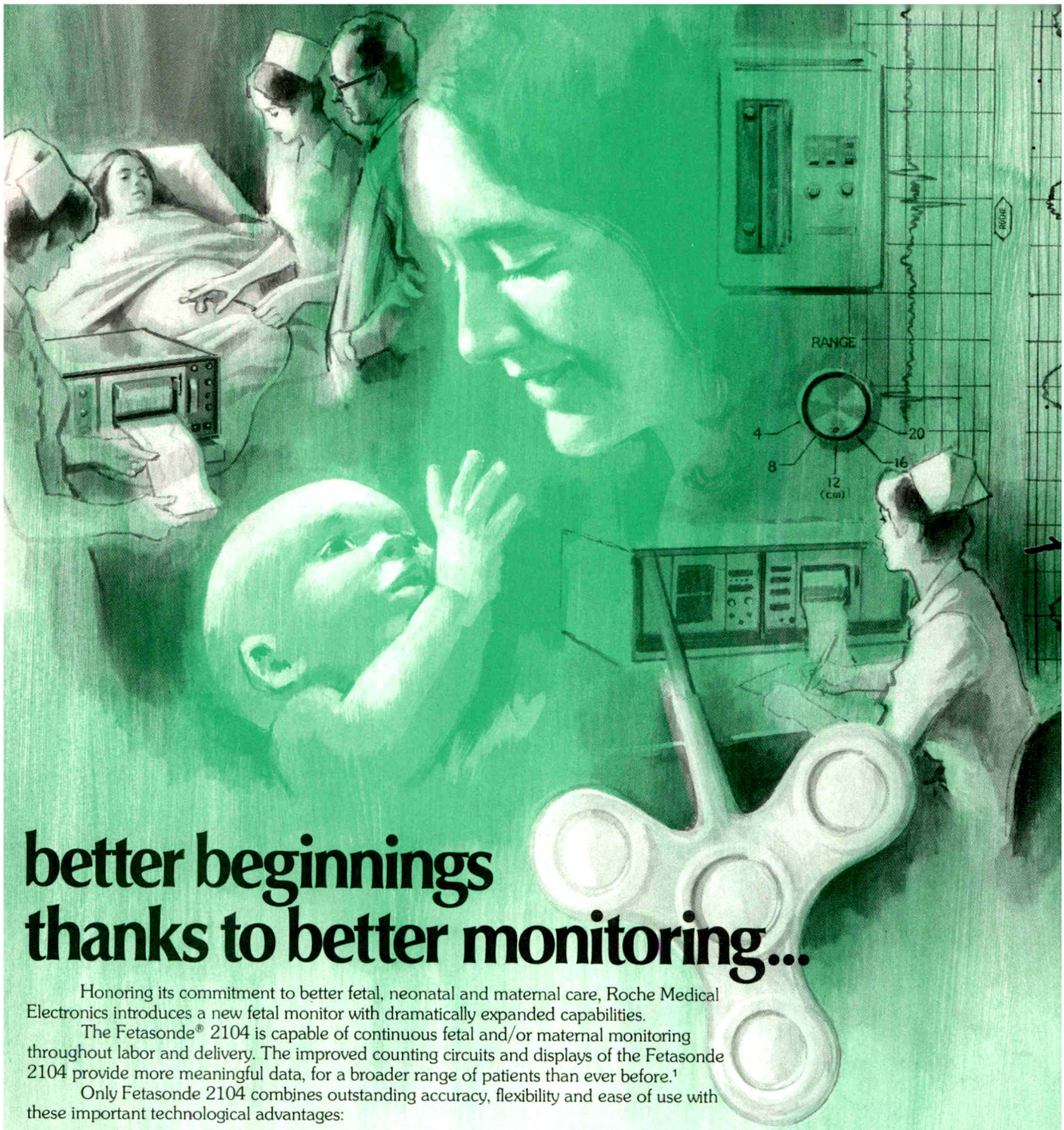
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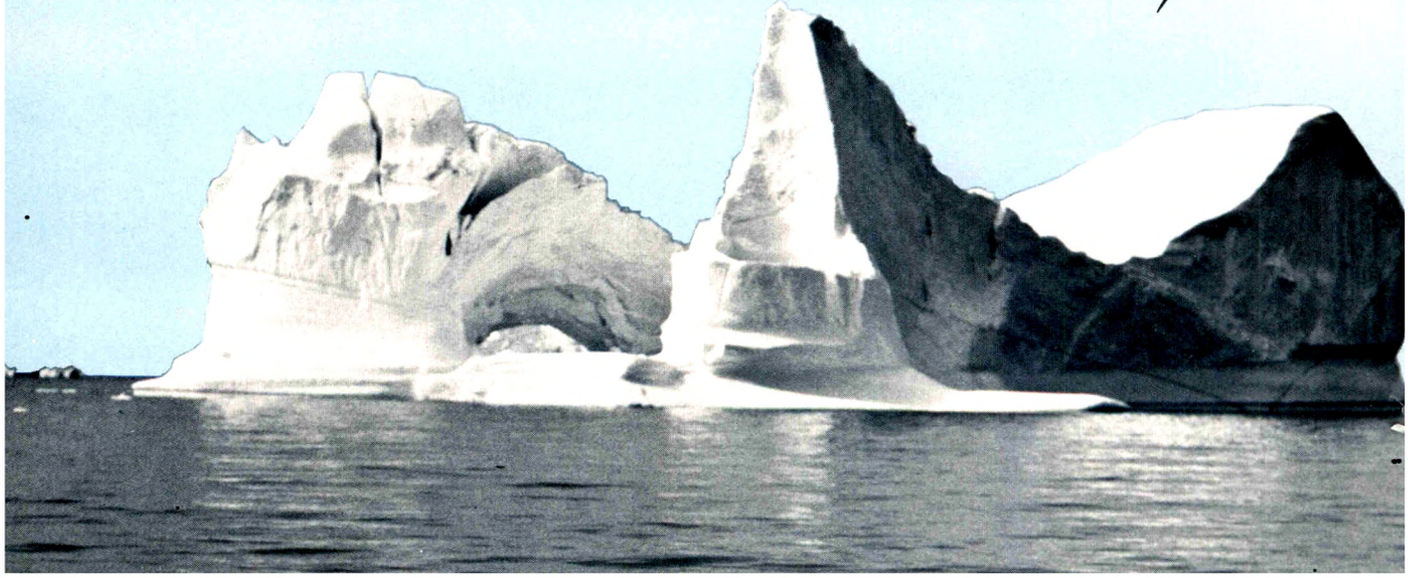
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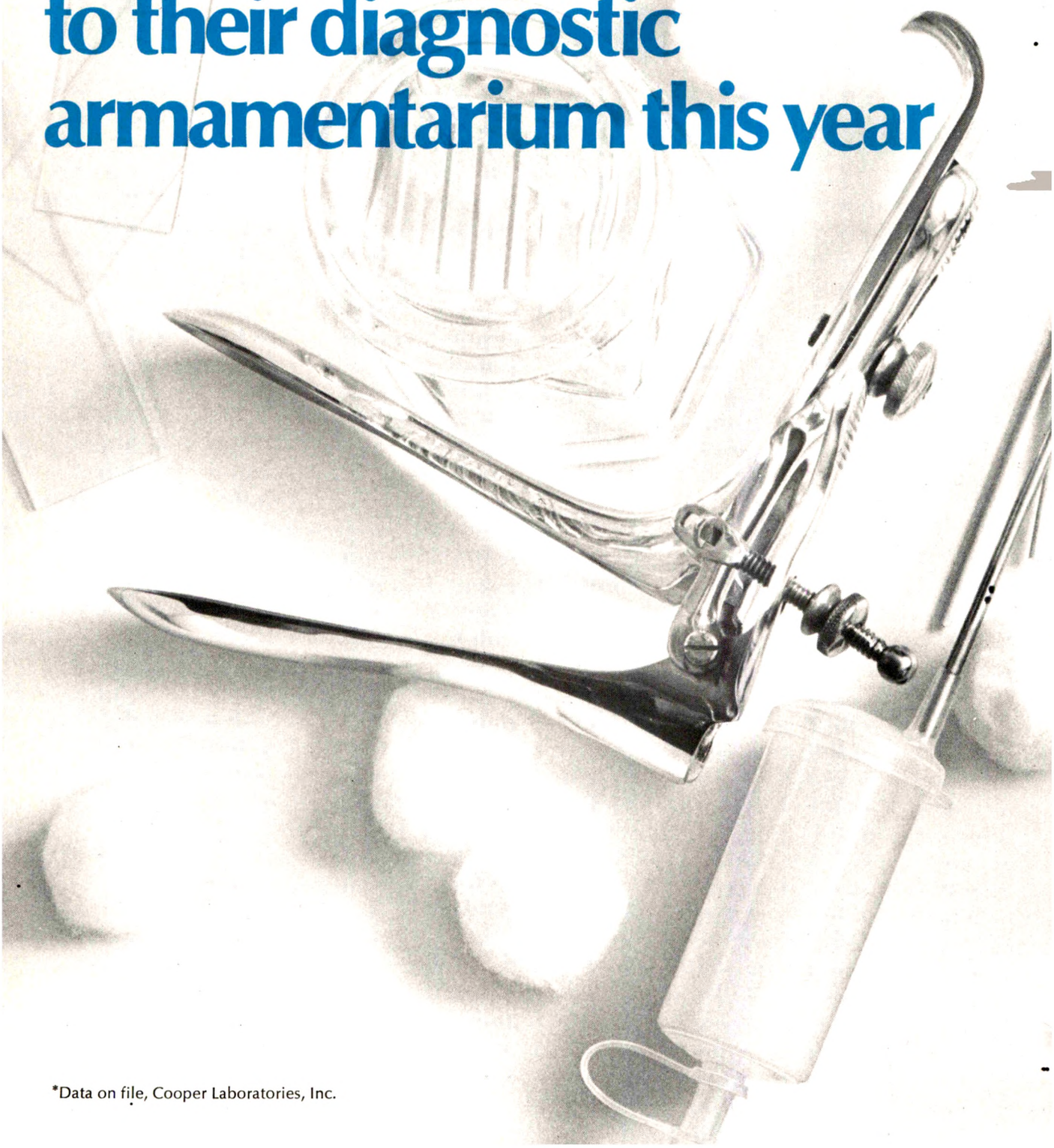
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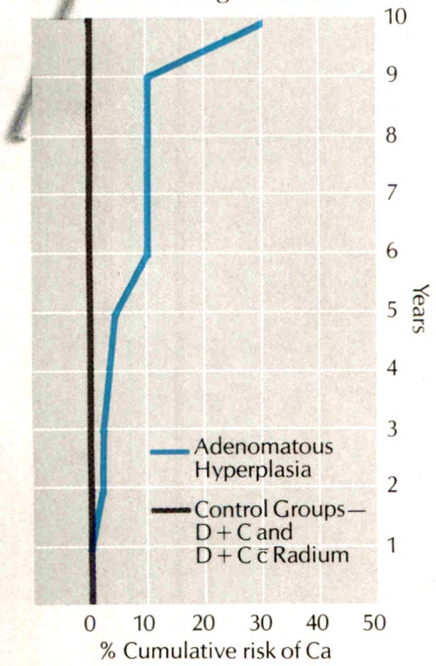


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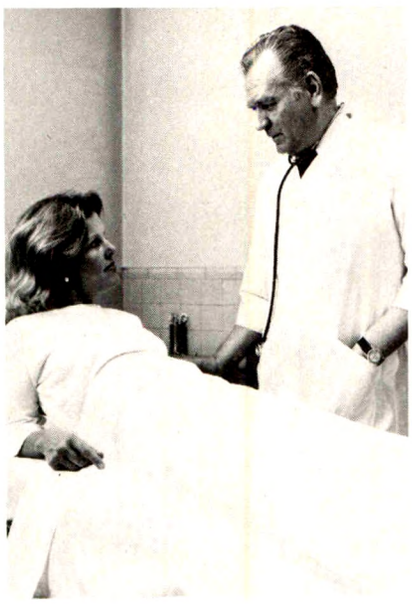
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Low dose oral contraceptive.

IN BRIEF:

SPECIAL NOTE: Oral contraceptives have been marketed in the United States since 1960. Reported pregnancy rates vary from product to product. The effectiveness of the sequential products appears to be somewhat lower than that of the combination products. Both types provide almost completely effective contraception.

An increased risk of thrombotic, thromboembolic and cerebral-vascular disease associated with the use of hormonal contraceptives has been shown in studies conducted in both Great Britain and the United States. Other risks, such as those of elevated blood pressure and reduced tolerance to carbohydrates, have been reported, and appropriate tests should be conducted to monitor these during oral-contraceptive therapy. Liver disease has also been reported, and the physician should be alert to its earliest manifestations.

Long-term administration of both natural and synthetic estrogens in subprimate animal species in multiples of the human dose increases the frequency for some animal carcinomas. These data cannot be transposed directly to man. The possible carcinogenicity due to the estrogens can neither be affirmed nor refuted at this time. Close clinical surveillance of all women taking oral contraceptives must be continued.

INDICATIONS: For oral contraception.

CONTRAINDICATIONS: 1. Thrombophlebitis, thromboembolic disorders, cerebral-vascular disease, or a past history of these conditions; 2. Markedly impaired liver function; 3. Known or suspected carcinoma of the breast; 4. Known or suspected estrogen-dependent neoplasia; 5. Undiagnosed abnormal genital bleeding; 6. Known or suspected pregnancy.

WARNINGS: 1. The physician should be alert to the earliest manifestations of thrombotic and thromboembolic disorders, thrombophlebitis, cerebrovascular disorders including hemorrhage, pulmonary embolism and retinal thrombosis. Should any of these occur or be suspected, the drug should be discontinued immediately.

Retrospective studies of morbidity and mortality in Great Britain and studies of morbidity in the United States have shown a statistically significant association between thrombophlebitis, pulmonary embolism and cerebral-vascular disease, occlusive or hemorrhagic, and the use of oral contraceptives. There have been three principal studies in Great Britain[1-3] leading to these conclusions and three[4-7] in this country. The estimate of the relative risk of thromboembolism in the study by Vessey and Doll[3] was about sevenfold, while the United States studies found relative risks of 4.4 to 11, meaning that the users are several times as likely to undergo thromboembolic disease without evident cause as non-users.

In May, 1974, the Royal College of General Practitioners in England[8] issued an interim report of its continuing large-scale prospective study comparing a user group to a non-user group. This study in its interim analysis states: "A statistically significant higher rate of reporting of cerebrovascular accidents in Takers is evident, but the numbers are too small to justify an estimation of the degree of risk." The study also reported a higher incidence of superficial and deep vein thrombosis in users as compared to non-users.

The Sartwell study[4] indicated that the risk did not persist after discontinuation of administration. Both the Sartwell and the Royal College study indicated that the degree of risk was not associated with duration of treatment.

In a collaborative American study[5,6] of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the relative risk of thrombotic stroke was 4.1 to 9.5 times greater in users than in non-users. A comparable estimate for hemorrhagic stroke was 2.0.

None of the American studies was designed to evaluate a difference between products. However, the Sartwell study[4] suggested that there might be an increased risk of thromboembolic disease in users of sequential products.

Other retrospective studies[9,10] have reported an increased risk of post-surgery thromboembolic complications in oral-contraceptive users. It has been recommended that therapy be discontinued at least one month prior to elective surgery.

2. Discontinue oral-contraceptive medication if there is: gradual or sudden, partial or complete loss of vision; proptosis or diplopia; onset or aggravation of migraine or development of headache of a new pattern which is recurrent, persistent or severe; papilledema; or any evidence of retinal-vascular lesions. 3. Fetal abnormalities have been reported to occur in the offspring of women who have taken progestogens and/or estrogens during pregnancy[11,12] The safety of LO/OVRAL® in pregnancy has not been demonstrated. Pregnancy should be ruled out before initiating or continuing the contraceptive regimen. Pregnancy should always be considered if withdrawal bleeding does not occur. 4. A small fraction of the hormonal agent in oral contraceptives has been identified in the milk of mothers receiving these drugs. The long-range effect to the nursing infant cannot be determined at this time. 5. Hepatic lesions (adenomas, hepatomas, hamartomas, regenerating nodules, etc.), occasionally fatal, have been reported in women on oral contraceptives. Such lesions may present as an abdominal mass or with the signs and symptoms of an acute abdomen. These lesions should be considered if the patient has abdominal pain or evidence of intra-abdominal bleeding. Such lesions have been reported in short-term as well as long-term users of oral contraceptives.

PRECAUTIONS: 1. A thorough history and physical examination should be performed before prescribing oral contraceptives and periodically during their administration and should include special reference to breasts and pelvic organs including Papanicolaou smears, since estrogens have been known to produce tumors, some of them malignant, in five species of subprimate animals. 2. Endocrine and possibly liver-function tests may be affected by treatment with LO/OVRAL® Therefore, if such tests are abnormal in a patient taking LO/OVRAL® it is recommended that they be repeated after the drug has been withdrawn for two months. 3. Under the influence of estrogen-progestogen preparations, pre-existing uterine fibromyomata may increase in size. 4. Because these agents may cause some degree of fluid retention, conditions which might be influenced by this factor, such as epilepsy, migraine, asthma, cardiac or renal dysfunction, require careful observation. 5. In breakthrough bleeding, and in all cases of irregular bleeding per vaginam, nonfunctional causes should be borne in mind. In undiagnosed bleeding per vaginam, adequate diagnostic measures are indicated. 6. Patients with a history of psychic depression should be carefully observed and the drug discontinued if the depression recurs to a serious degree. 7. Any possible influence of prolonged LO/OVRAL® therapy on pituitary, ovarian, adrenal, hepatic or uterine function awaits further study. 8. A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. The mechanism of this decrease is obscure. For this reason, diabetic patients should be carefully observed while receiving LO/OVRAL® therapy. 9. The age of the patient constitutes no absolute limiting factor, although treatment with LO/OVRAL® may mask the onset of the climacteric. 10. The pathologist should be advised of LO/OVRAL® therapy when relevant specimens are submitted. 11. Susceptible women may experience an increase in blood pressure following administration of contraceptive steroids. 12. Cholestatic jaundice has been reported in users of oral contraceptives. If this occurs, LO/OVRAL® should be discontinued. This condition is more likely to occur in patients who have experienced cholestatic jaundice of pregnancy. Patients with a history of cholestatic jaundice of pregnancy should be carefully observed during LO/OVRAL® therapy.

ADVERSE REACTIONS OBSERVED IN PATIENTS RECEIVING ORAL CONTRACEPTIVES: A statistically significant association has been demonstrated between use of oral contraceptives and the following serious adverse reactions: thrombophlebitis, pulmonary embolism, cerebral thrombosis and hemorrhage, gall bladder disease.

Although available evidence is suggestive of an association, such a relationship has been neither confirmed nor refuted for the following serious adverse reactions: neuro-ocular lesions, e.g., retinal thrombosis, optic neuritis and hepatic lesions with or without intra-abdominal bleeding.

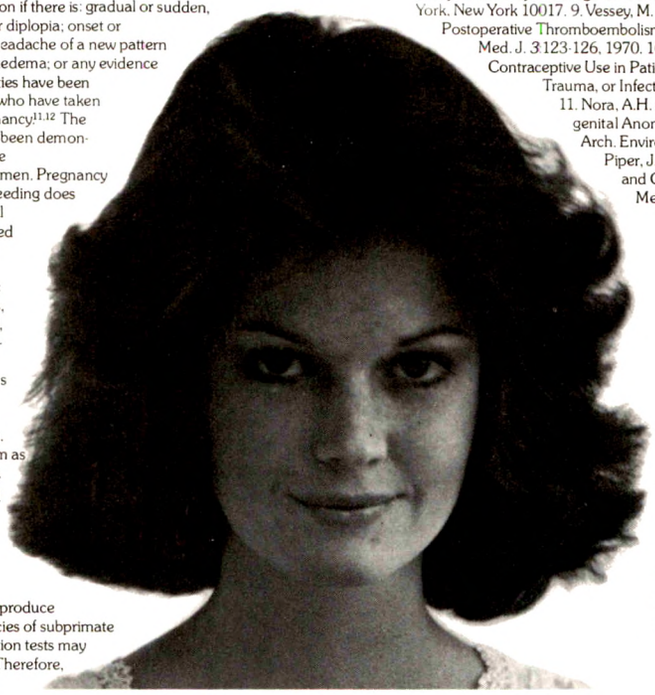
The following adverse reactions are known to occur in patients receiving oral contraceptives: nausea, vomiting, gastrointestinal symptoms (such as abdominal cramps and bloating), breakthrough bleeding, spotting, change in menstrual flow, amenorrhea during and after treatment, edema, chloasma or melasma, which may persist, breast changes: tenderness, enlargement and secretion, change in weight (increase or decrease), changes in cervical erosion and cervical secretions, suppression of lactation when given immediately post-partum, cholestatic jaundice, migraine, rash (allergic), rise in blood pressure in susceptible individuals, mental depression.

Although the following adverse reactions have been reported in users of oral contraceptives, an association has been neither confirmed nor refuted: anovulation post treatment, which tends to occur more frequently in women with a history of menstrual disorders, premenstrual-like syndrome, changes in libido, changes in appetite, cystitis-like syndrome, headache, intolerance to contact lenses, nervousness, dizziness, fatigue, backache, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, itching and vaginitis.

The following laboratory results may be altered by the use of oral contraceptives: hepatic function; increased sulfobromophthalein retention and other tests; coagulation tests; increase in prothrombin, Factors VII, VIII, IX and X; decrease in antithrombin III; increase in platelet aggregability; thyroid function: increase in PBI, and butanol extractable protein-bound iodine and decrease in T^3 uptake values; metyrapone test; and pregnanediol determination.

NOTE: For more specific details on LO/OVRAL, refer to package insert.

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CLINICAL OPINION

This section reports opinion on the handling of clinical situations, i.e., the clinical diagnosis and management of certain disease entities. Papers should range from eight to twenty typed pages, including illustrations, tables, and figures which clarify the author's management. References are limited to six citations. Mail to Frederick P. Zuspan, M.D., Editor.

Management of the hyperextended fetal head

E. DAW

Dundee, Scotland

Hyperextension of the fetal head in utero is readily detectable by roentgenography and ultrasound scan. Management is determined by presentation of the fetus. There are three types of presentations: face presentation, star-gazing breech presentation, and the flying fetus presentation. Undiagnosed hyperextension of the fetal head is frequently associated with cervical cord injury.

HYPEREXTENSION of the fetal head in utero gives rise to characteristic roentgenographic and ultrasound scan appearances, and the management of the individual case will depend initially upon this diagnosis and subsequently depending upon several other factors. The presentation of the fetus is important in this condition: If the head is presenting, the appearances are those of "face" presentation; if the breech is presenting, the appearances are those of a "star-gazing" breech presentation; if the fetus is lying transversely, the appearance is that of the "flying fetus" presentation. These three presentations are illustrated graphically in Figs. 1 to 3. In this paper I wish to review and discuss recent changes in management of these conditions.

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Classification

Face presentation (Fig. 1) may be further subdivided into mentolateral, mentoposterior, and mentoanterior presentations, depending upon the relationship of the chin of the fetus to the pelvic inlet. As the head is not relative to the pelvis with a star-gazing breech presentation (Fig. 2), no subdivision of this group is made, and the breech presentation is classified as usual, depending upon whether the legs are extended or flexed. Hyperextension of the fetal head with the flying fetus (Fig. 3) is associated with extension of the fetal arms and spine and partial extension of the fetal legs. This attitude of the fetus is quite characteristic and obviously different from "transverse lie" of the fetus with

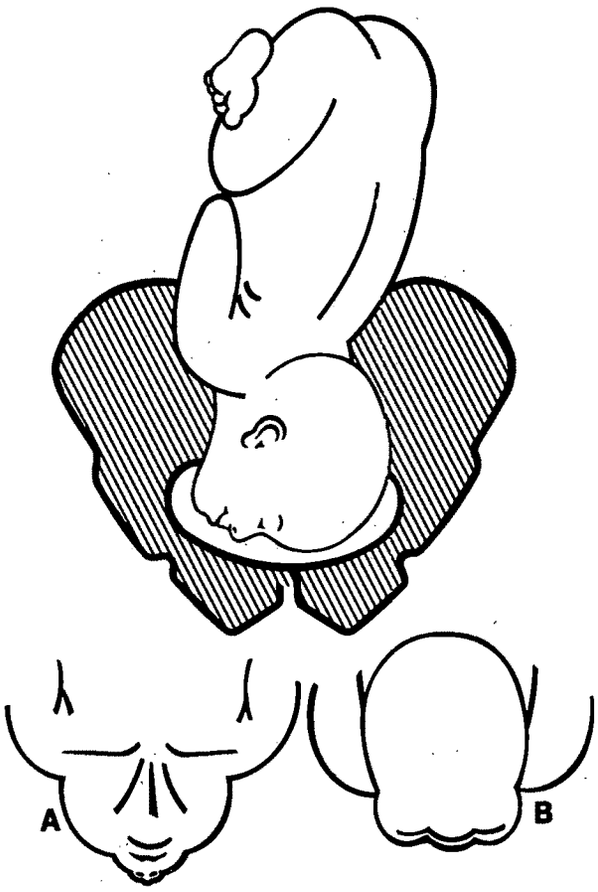


Fig. 1. Face presentation (mentolateral) with mentoanterior *(A)* and mentoposterior *(B)* variations.

generalized flexion of the fetus and the back either upward or downward.

Incidence

Face presentation has the commonest incidence, occurring approximately in one of every 500 hospital cases. The star-gazing breech and the flying fetus presentations occur so infrequently that one cannot give an accurate incidence.

Complications

The relationship between intrauterine hyperextension of the fetal head and subsequent cervical cord injury during vaginal breech delivery is now established.[3] The usual site of injury is the lower cervical region, and the injury frequently escapes notice because of no associated bony damage. The three clinically recognizable groups of injury are: (1) either stillbirth or sudden death of the infant soon after delivery; (2) survival of the infant for a short period, in shock, with respiratory depression and secondary changes in the lungs similar to those with hyaline membrane disease; (3) survival of the infant with

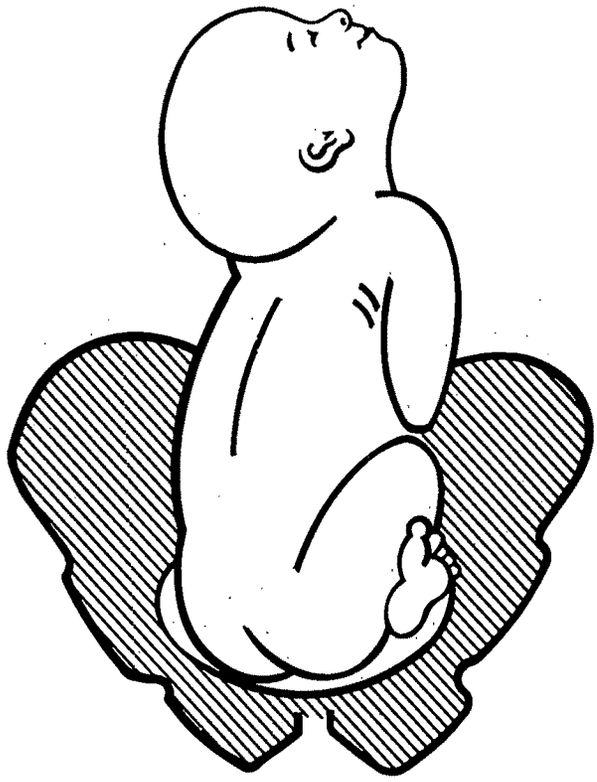


Fig. 2. Star-gazing breech presentation.

transient or permanent paralysis and spasticity. Bhagwanani and associates[1] thought that even with the use of average traction the sudden extreme flexion of the head and cervical spine, which is so much a part of vaginal breech delivery, was sufficient to damage the vulnerable spinal cord. The resulting flexion injury is similar to the rotational injury occasionally seen with forceps delivery.[6] The lesions are similar macroscopically and microscopically with no obvious bony injury, but the rotational injury is usually at a higher level (at the level of the second cervical vertebra). A recent review of the literature[3] shows the degree of protection afforded the infant in the star-gazing breech presentation when delivery is effected by cesarean section.

Diagnosis

Hyperextension of the fetal head is often not diagnosed until labor has commenced; however, in many cases, some abnormality was suspected during the antenatal period but this was not followed up by radiography or ultrasound scan. A high presenting part or a persistent abnormality, such as a transverse lie on abdominal palpation, merits further investigation such as ultrasound scan or x-ray in the later weeks of pregnancy. During labor, transverse lie or a persistently high presenting part on abdominal examination also merits such investigation.

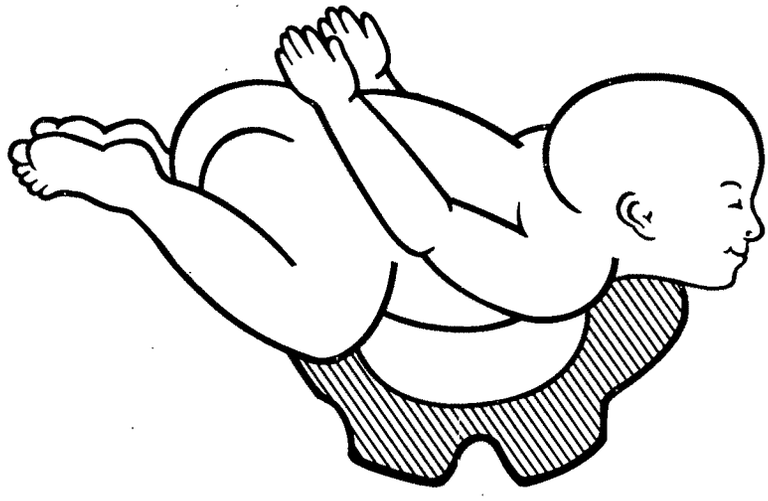


Fig. 3. Flying fetus presentation.

On vaginal examination in the later weeks of pregnancy the high presenting part may not be reached, but classically, with a patient in labor, the forewaters are sausage-shaped with the presenting part high and indistinct.

Ultrasound scan has an advantage over radiography in that neck tumors may be detected, but, as Bhagwanani and associates[1] concluded, "Radiologic evidence of hyperextension of the fetal head . . . after labor is established seems to be an indication for cesarean section unless there is gross fetal deformity. The remote possibility of an abnormality that is radiologically undetectable should not discourage an obstetrician."

Management of delivery

As *face presentation* is more common than the other two, most units will have a policy for dealing with this condition; therefore, I will only list some principles of management of delivery.

1. Exclusion of fetal abnormality, particularly anencephaly, by x-ray or ultrasound scan is of paramount importance. If the fetus appears normal, primary face presentation may be left alone until labor starts.

2. Once labor starts, if the face presentation persists and if pelvic contraction is suspected or if the fetus is unduly large, or if there is a suspicion of placenta previa or any other condition putting the fetus "at risk," cesarean section delivery is indicated.

3. In the remaining cases of face presentation, the position of the chin as the head descends into the pelvis then becomes important; as a rule, mentoposterior face presentation undoubtedly indicates cesarean section.

4. In areas where the perinatal mortality rate is low because there are facilities for rapid intervention, *heroic* obstetric interference, with its high potential injury to the fetus, is avoidable. However, manipulative procedures such as Thorn's maneuver, or internal version and breech extraction may be necessary where facilities for rapid intervention are not available.

In the absence of evidence of gross fetal abnormality, the *flying fetus* presentation is best managed by elective cesarean section. Prolapse of the cord during labor is an obvious complication in this condition, but, should full dilatation of the cervix occur, the problem of reaching the extended lower limbs for internal version is also real.

Most recent reports and reviews have shown that after labor is established in the absence of evidence of gross fetal abnormality the *star-gazing breech* presentation is best managed by elective cesarean section. Taylor[7] has pointed out that the safest method for delivery of the term infant presenting as a breech is by cesarean section; this is true for the infant in a star-gazing breech presentation even before term.

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GYNECOLOGY

A comparison between gonadotropin radioimmunoassays with the use of 48 hour and six-day incubation methods

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A double-antibody radioimmunoassay for human follicle-stimulating hormone (FSH) and human luteinizing hormone (LH) which can be carried to completion in 48 hours was compared with the conventional six-day double-antibody radioimmunoassay. The bound-over total (B/T) dose response curves for the 48 hour assay were comparable in slope and dose range with the six-day assay and the four per cent average within-assay and the nine per cent between-assay coefficients of variation for the 48 hour assay were comparable with the variation observed in the conventional six-day assay. Maximum variation among identical serum samples assayed in both systems was 15.3 per cent so that quantitative results obtained with either assay system were comparable.

IN THE MOST commonly used double-antibody radioimmunoassay method, an immunoprecipitating serum or second antibody to precipitate soluble antigen-antibody complexes is used. This technique is stable, sensitive, precise, and offers reliability and reproducibility.[1] It is known that a period of incubation of sufficient length is required to ensure that the reaction reaches equilibrium. This has been shown to be a matter of days, and not hours, to ensure completion.[1] Results can be obtained more rapidly but only at the expense of using considerably higher concentrations of antibody to bind labeled hormone, thus compromising sensitivity.[1] This appears to be true if the complete reaction is carried out at four degrees C.

Recently a radioimmunoassay with the use of the double-antibody technique has been reported which requires only 36 to 48 hours for completion.[2, 3] This method was first described for the human chorionic gonadotropin–B (HCG-B) subunit assay but has also been used successfully for several years for human follicle-stimulating hormone (FSH) and human luteinizing hormone (LH).[4]

Because of the consistently sensitive and reproducible results obtained with a HCG-B subunit assay, it was decided that the shorter incubation methodology for FSH and LH assays should be used. A prospective study was initiated with half of the assays with the use of the 48 hour incubation method, and half with the use of the six-day incubation.

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Material and methods

Hormone preparations.

Human chorionic gonadotropin. Highly purified HCG was obtained from Serono Pharmaceuticals (Rome, Italy). This purified HCG had a biological activity of 12,000 to 18,000 I.U. per milligram and a radioimmunologic activity of 6,000 to 7,000 I.U. per milligram Second International Reference Preparation of HCG.

Anti-HCG antiserum was generated in rabbits and was found to be sufficiently specific for HLH for purposes of radioimmunoassay.

Human follicle-stimulating hormone. Purified pituitary FSH (LER-1366) for purposes of iodination was provided by the National Pituitary Agency through the Hormone Distribution Officer, National Institute of Arthritis and Metabolic Disease.

Anti-FSH Batch No. 3 was obtained through the courtesy of the National Pituitary Agency.

The standard curve for each assay was made up from a commercial preparation of human menopausal gonadotropin* (HMG) previously assayed against the Second International Reference Preparation of Human Menopausal Gonadotropin and consisted of eight serial dilutions from 5 mI.U. per milliliter to 640 mI.U. per milliliter.

Iodination. The iodination procedure utilized has been described previously.[3] Fresh columns were always prepared on the day of iodination. A 5 × 0.5 cm. column bed of Biogel P-10 (Bio Rad Labs, Richmond, California) was prepared by washing three to four times with 0.01M phosphate-buffered saline (PBS), followed by 1.5 ml. of two per cent bovine serum albumin (Sylvana Co., Milburn, New Jersey). A final washing with two columns of 0.01M PBS was done before the column was ready for the iodinated mixture. All iodinations were carried out at room temperature.

Human chorionic gonadotropin. Two millicuries of low–specific activity $Na^{125}I$ (Amersham/Searle, Chicago, Illinois) was added to 4 μg of highly purified HCG dissolved in 0.025 ml. of 0.4M Na phosphate buffer pH 7.5. Chloramine-T (0.01 ml. of a 25 mg. per 10 ml. solution of 0.01M PBS) was added to the hormone. Sodium metabisulfite (0.025 ml. of a 25 mg. per 10 ml. solution of 0.01M PBS) was used to stop the reaction after two minutes. After ten seconds of mixing the mixture was added to the column and collected in 0.5 ml. aliquots. To the peak tube was added 0.05 ml. of two per cent bovine serum albumin in 0.01M PBS and 0.5 ml. of 0.01M PBS. The iodinated hormone was stored at 4 degrees C. Specific activities for HCG ranged from 140 to 240 μc per microgram.

Human follicle-stimulating hormone. Low–specific activity $Na^{125}I$, one millicurie was added to 2 μg of FSH (LER-1366) dissolved in 0.025 ml. of 0.4M Na phosphate buffer. Chloramine-T and sodium metabisulfite were used in the same concentrations described for HCG except the reaction mixture was stopped after 20 seconds. Specific activities for FSH ranged from 130 to 224 μc per microgram.

Radioimmunoassay. The following reagents, added to a 10 × 75 mm. disposable glass tube to make a final volume of 1 ml., were used in the radioimmunoassay for LH: (1) buffer containing 0.01M PBS and one per cent egg whites; (2) 0.2 ml. of serum to be assayed or 0.2 ml. of standard in buffer; (3) 0.2 ml. of anti-HCG antisera (1:100,000 final tube dilution) in 0.05 M ethylenediamine tetra-acetic acid (EDTA), 0.01M sodium phosphate, 0.14M sodium chloride, pH 7.0, and 1:400 normal rabbit serum; (4) 0.1 ml. of ^{125}I-HCG containing 0.1 μg of hormone with counts ranging from 14,000 to 24,000 c.p.m. depending on the specific activity, (5) 0.2 ml. of sheep anti-rabbit gamma globulin (RGG).

The reagents used in the radioimmunoassay for FSH were the same as those used in the assay for LH except that 0.2 ml. of anti-FSH antisera (1:15,000 final tube dilution Batch No. 3 NPA) and 0.1 ml. of ^{125}I-FSH (13,000 to 22,000 c.p.m.) were used.

Standard curves for all the assays contained the appropriate amount of antigenic hormone in one per cent egg white buffer but contained no serum or plasma.

Procedure.

48 Hour assay. Antisera, iodinated hormone, buffer, and the serum to be assayed were incubated in a 37 degree C. water bath for two hours. After removal from the water bath the samples were incubated at four degrees C. for 22 hours. Second antibody (sheep antirabbit gamma globulin) was added and the mixture incubated at four degrees C. for an additional 24 hours to achieve separation of bound antibody from free labeled hormone. Tubes were centrifuged at 3,000 r.p.m. for 20 minutes on a Sorval RC-2 centrifuge, and the supernatant was aspirated by suction. The precipitate was counted in an automatic well-type gamma counting system (Searle Analytic, Chicago, Illinois).

Six-day assay. The method used for this study was based on the procedure as described by Midgely and Jaffe.[5, 6] Antisera, buffer, and the serum to be assayed were incubated at four degrees C. for 24 hours. Iodinated hormone was added and the mixture

*Pergonal, Serono Laboratories, Boston, Massachusetts.

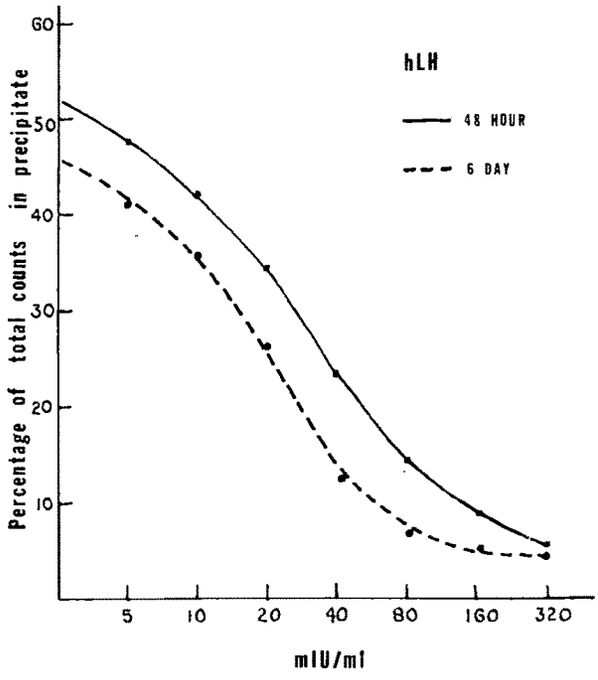


Fig. 1. Comparison of standard curves for LH with the use of a 48 hour versus a six-day incubation methodology. First antibody was used at 1:100,000 final tube dilution for both systems.

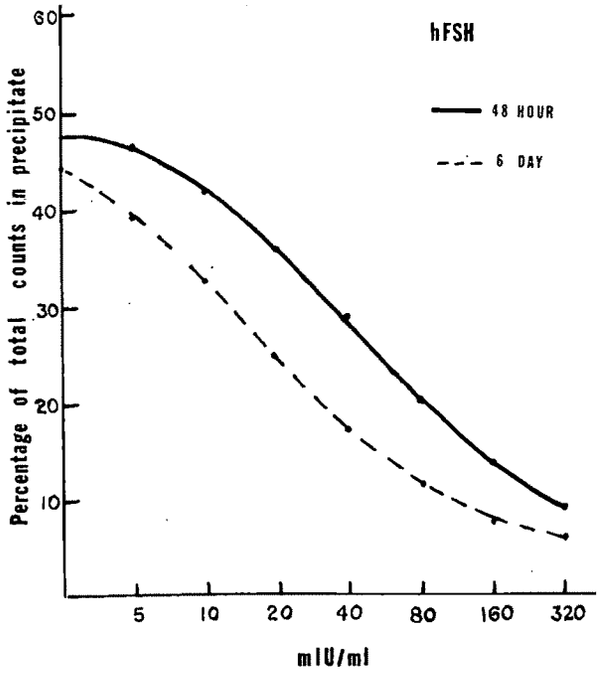


Fig. 2. Comparison of standard curves for FSH with the use of a 48 hour versus a six-day incubation methodology. First antibody was used at 1:15,000 final tube dilution for both systems.

incubated at four degrees C. for 24 hours. Second antibody was then added and incubation carried out at four degrees C. for 96 hours. Tubes were then centrifuged, aspirated, and counted by use of the procedure described for the 48 hour assay.

In both the 48 hour assay and the six-day assay, the assay tubes were identical with respect to antibody dilution, buffer solutions, and iodinated hormone.

Analysis of radioimmunoassay results. All radioimmunoassay data were analyzed by a computer program (Lee, E. Y., and Kosasa, T. S., in preparation). Analysis began with the percentage binding being expressed as: Y = B (Counts bound in the presence of labeled and unlabeled ligand) − background counts, divided by B_0 (Counts bound in the presence of labeled ligand alone) − background counts. The relation between Y and the log (dose) for the standard curve was analyzed through two different approaches. The first was the linear regression of logit (Y) on the log dose and the second was a curvilinear fitting of Y as a quartic polynomial of the log (dose). The standard error of fitting was then calculated for each approach and the program automatically used the approach which was associated with the least amount of error. An inverse transformation was performed on the surviving functional relation to obtain log (dose) as a function of Y. Unknown samples were then claculated from this standard curve and the results expressed in milli-international units per milliliter.

Results

In a six-month period over 2,000 assay tubes for FSH and 2,000 assay tubes for LH were determined with the use of a 48 hour incubation method. During the same time period a comparable number of assay tubes were determined for FSH and LH with the use of the six-day incubation method. A consistent finding in both the FSH and LH assays was the higher binding of tracer antigen observed with the same dilution of antisera in the 48 hour assay. The bound counts in the LH zero standard tubes B_0 (amount bound in the absence of unlabeled antigen) in the 48 hour assays were 4.2 to 8.6 per cent higher than the zero standard tubes in the comparison six-day assays. The zero standard tubes in the 48 hour FSH assays were two to 12.2 per cent higher than those in the comparison of six-day assays (Figs. 1 and 2).

The within-assay coefficient of variation for the 48 hour assay was four per cent and the between-assay coefficient of variation nine per cent as compared with three per cent within assay and seven per cent between assay for the six-day assay.

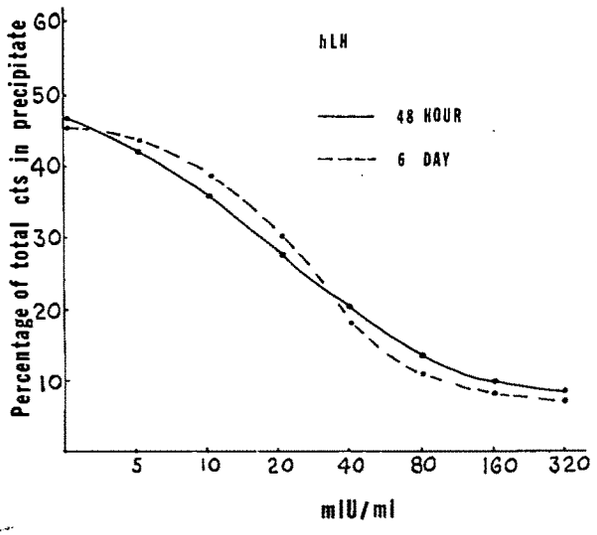


Fig. 3. Comparison of standard curves for LH with the use of a 48 hour versus a six-day incubation methodology. First antibody was used at 1:150,000 for the 48 hour assay and 1:100,000 for the six-day assay.

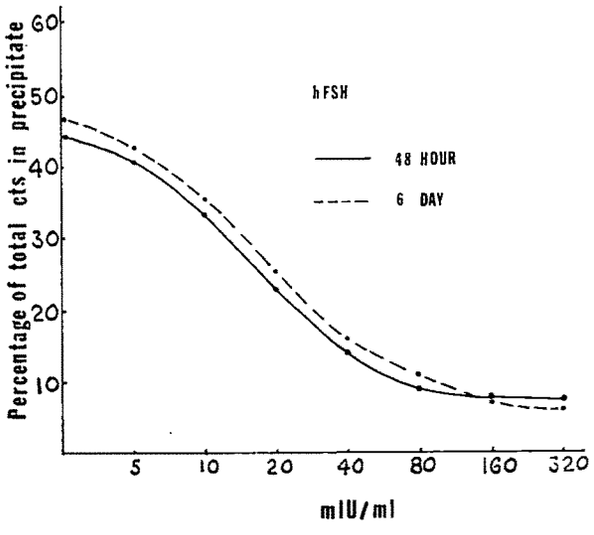


Fig. 4. Comparison of standard curves for FSH with the use of a 48 hour versus a six-day incubation methodology. First antibody was used at 1:20,000 for the 48 hour assay and 1:15,000 for the six-day assay.

Eight identical serum samples from a post-menopausal pool were included in every assay to compare any quantitative differences between the 48 hour and six-day incubation methods. The average of the pool for LH was 90.6 mI.U. per milliliter and that for FSH was 112.6 mI.U. per milliliter. Differences among these samples in the 48 hour and six-day assay systems were 2.9 mI.U. per milliliter to 12.2 mI.U. per milliliter in ten consecutive assays for LH and 1.0 to 11.4 mI.U. per milliliter in ten consecutive assays for FSH.

Comment

Although it is known that some antibodies lose effectiveness when incubated at 37 degrees C., none of the antibodies used in this study appeared to be affected when incubated at this temperature. Commercial anti-HCG and anti-FSH (Calbiochem, La Jolla, California) appeared to work equally as well in both the 48 hour and six-day assays.

The parallelism between the standard curves employing the 48 hour and six-day incubation method for FSH and LH appear to indicate similar immunologic behavior for the two systems. This parallelism has been observed in a radioimmunoassay which carries out the complete reaction at 37 degrees C.[7] The slight increase in binding of the standard curves with the use of the 48 hour incubation method suggests that the first antibody could be used at a slightly greater dilution with a subsequent improvement in sensitivity when measuring sera with low levels of hormone. By use of a final anti-HCG dilution of 1:150,000 in the 48 hour assay and comparing it to the six-day assay with a dilution of 1:100,000, the standard curves are virtually indistinguishable (Fig. 3). Similar results have been shown when the anti-FSH has been reduced to a final dilution of 1:20,000 in the 48 hour assay (Fig. 4).

In this comparison study anti-RGG from our own sheep was used diluted 1:8 in PBS buffer. It was found that anti-RGG from commercial sources could be used at greater dilutions (Serono Pharmaceuticals, Rome, Italy, 1:25, and Antibodies Incorporated, Davis, California 1:32). When these commercial sources of anti-RGG were used, the precipitates in the B_0 and standard tubes were almost nonexistent in the 48 hour assay. As a result there was a frequent loss in precipitated counts from aspiration errors. In order to create an adequate precipitate to facilitate aspiration when anti-RGG from these sources was utilized, 0.1 ml. of normal sheep serum was added to the background, B_0, and standard tubes. Presently the assay tubes for the 48 hour incubation method are centrifuged at 5,000 r.p.m. for seven minutes without any appreciable differences in results.

It has been shown that identical serum samples may have differences in the quantitative results when measured by different laboratories with the use of the same standard, antisera, and incubation methods. Even greater differences in the quantitative results have been shown when the methodologies are altered.[8] Identical serum samples assayed in both the 48 hour

and six-day assays showed quantitative differences but were within an acceptable range of variation. Although the maximum coefficient of variation of 13.6 per cent for LH and 15.3 per cent for FSH between the two assay systems was greater than the maximum between-assay variation of 12 per cent for the 48 hour assay and 10.2 per cent for the six-day assay, it appeared reasonable considering the differences in methodology. The within-assay and between-assay coefficients of variation for both the 48 hour and six-day assay were comparable to the variation for the double antibody radioimmunoassay system reported by others.[9-12].

From this study it has been shown that quantitative results obtained in the 48 hour assay were comparable with those obtained in the six-day assay. The added advantage of providing a result in 48 hours rather than one week makes this assay well suited for a clinically oriented laboratory.

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The Copper T 220C: A new long-acting copper intrauterine contraceptive device

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The Copper T(TCu) intrauterine contraceptive device (IUD) has been found to be an effective method of contraception but has the disadvantage of needing periodic replacement due to loss of copper through dissolution and fragmentation of the copper wire. Modification of the Copper T to overcome this disadvantage involved substitution of copper sleeves for wire on the vertical arm and the addition of copper sleeves to the horizontal arms to give an effective surface area of 220 sq. mm. of copper and an estimated life span of 20 years. A randomized comparative study was undertaken in 999 women to determine if the new model performed as well as the wire-wound Copper T 300. Net cumulative first-event rates at one year were as follows: TCu 220C—pregnancy 0.9, expulsion 3.7, removal for pain or bleeding 14.4; TCu 300—pregnancy 1.1, expulsion 3.1, removal for pain or bleeding 9.3. None of the differences in event rates were significant. If long-term follow-up confirms these findings, this new long-lasting copper intrauterine device could become the IUD of choice for all women.

MANY REPORTS FROM this clinic, as well as others, have shown that the Copper T(TCu) intrauterine contraceptive device (IUD) is an effective and well-tolerated contraceptive method for both nulliparous and multiparous women.[1–3]

In addition to the extensive clinical studies of the TCu 200, various modifications in the amount of copper and its position upon the plastic have been developed and tested clinically. The TCu 300 has approximately 300 mm.^{2} copper wire wound around the vertical arm, while the TCu 380A has two 30 mm.^{2} copper sleeves placed on the horizontal arm in addition to 300+ mm.^{2} copper on the vertical arm. The disadvantage of the use of copper wire is its relatively short duration of action. Devices with copper wire need to be replaced at intervals of about three years.

To overcome this disadvantage, the International Committee for Contraception Research of The Population Council devised a new modification of the TCu, the Copper T 220C. This device has five solid copper sleeves on the vertical arm, as well as two copper sleeves on the horizontal arm of the T-shaped plastic device for a total of seven sleeves with a surface area of 220 mm.^{2} and an estimated duration of effectiveness of more than 20 years.

In order to compare the performance of this long-lasting copper IUD with the wire-wound model, a randomized comparative study of the TCu 220C and the TCu 300 was performed.

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Material and methods

Between January and July, 1973, 999 TCu devices were inserted into all women attending the Family Planning Clinic of Women's Hospital who were willing to use an investigational IUD and were able to return for follow-up. The device to be inserted was chosen on the basis of the last digit of the patient's previously assigned hospital number. Patients with odd numbers

Table I. Mean and range of various aspects of total cohort data of two groups

Data	*TCu 300*	*TCu 220C*
Number inserted	516	483
Age	23.1 (14-42)	22.7 (15-45)
Gravidity	1.05 (0-9)	1.20 (0-6)
Parity	0.55 (0-9)	0.48 (0-5)
Nulliparous women	363 (70.3%)	337 (69.8%)

Table II. Mean and range of cohort data of nulliparous women in two groups

Data	*TCu 300*	*TCu 220C*
Number inserted	363	334
Age	22.3 (14-40)	22.2 (15-39)
Gravidity	0.38 (0-6)	0.34 (0-3)
Parity	0	0

received a TCu 220C. Those with even numbers received a TCu 300. A total of 463 women received the 220C and 516 the 300. All devices were inserted by family planning clinic staff physicians with the use of a modified loop inserter and push-in technique.[4]

As would be expected from this randomization, the populations using each device were similar in age, gravidity, parity, and per cent of nulliparous women (Table I). Separate analysis of nulliparous women and multiparous women separately also revealed that the groups using each device were comparable (Tables II and III). Event rates during the first 12 months' experience were calculated by life-table analysis with a computer program.[5]

Results

There was no difference noted in ease of insertion or postinsertion pain between the two devices. First segment net cumulative termination rates for the first year of use for each device are summarized for all patients in Table IV, for nulliparous patients in Table V, and for multiparous patients in Table VI. For each event rate, the 95 per cent confidence intervals of the standard error of the mean overlapped, indicating no significant difference between the two devices. The pregnancy rates at one year were about one per cent for each device. Pregnancy rates were slightly though not significantly higher among multiparous women. About 70 per cent of the nulliparous patients had never been pregnant, and a certain percentage of these women were probably infertile.

Expulsion rates at one year were less than 4.0 per 100 women in both groups with little difference between devices or between nulliparous and multiparous women. The rate of removals for pain and/or bleeding was higher for the TCu 220C than for the TCu 300, but the difference was not significant. Rates of removals for other medical reasons, planned pregnancy, and other personal reasons were also similar for the two devices.

Table III. Mean and range of various aspects of cohort data of multiparous women in two groups

Data	*TCu 300*	*TCu 220C*
Number inserted	153	146
Age	25.1 (15-42)	24.1 (15-45)
Gravidity	2.3 (1-9)	2.0 (1-6)
Parity	1.9 (1-9)	1.6 (1-5)

Over-all continuation rates at the end of one year were also similar for both devices, with rates of 79.7 and 74.9 per 100 users calculated for the TCu 300 and TCu220C, respectively. This difference in continuation rates was also not significant.

Comment

The major disadvantage of the Copper T with wire winding is the need for periodic replacement. This arises because the copper undergoes gradual dissolution in an uneven manner so that breaks in the copper wire can occur. The TCu 220C was developed to overcome this disadvantage. By substituting sleeves for wire, a much greater effective reservior of copper is achieved and the possibility of wire breakage is obviated. The TCu 220C also includes copper sleeves on the horizontal arms, which decreases the chance of fundal pregnancy because of the presence of copper in that area. This is especially important when there is downward displacement or partial expulsion of the device.

The TCu 200 device has been estimated to lose between 25 and 50μg per day of copper,[6] and Tatum[7] has recommended that these devices be replaced at three-year intervals based on the actual amount of copper remaining on the device and the continued contraceptive effectiveness at two years. By substituting sleeves for wire and assuming the rate of release of copper is similar per unit surface area for both sleeves and wire, the copper on the TCu 220C is estimated to last more than 20 years. This clinical trial has shown that enough copper is released to maintain comparable contraceptive effectiveness at least through the first year.

The Copper T has already been shown to have two major advantages in comparison with other plastic IUD's: (1) The TCu is easier to insert and better

Table IV. Net event rates per 100 women at one year

Rates	*TCu 300*	*TCu 220C*
Event		
Pregnancy	1.1 (0.3-2.4)	0.9 (0.2-2.2)
Expulsions	3.1 (1.5-4.6)	3.7 (1.9-5.5)
Removals		
Pain/bleeding	9.3 (6.7-12.0)	14.4 (11.1-17.7)
Other medical	2.1 (1.0-3.8)	0.9 (0.2-2.2)
Planned pregnancy	2.7 (1.2-4.2)	3.1 (1.5-4.7)
Other personal	2.1 (1.0-3.8)	2.2 (1.1-4.0)
All causes	20.3 (16.7-24.0)	25.1 (21.1-29.2)
Continuation	79.7	74.9
Number enrolled	516	483
Woman months	5,205	4,775
Lost to follow-up	9%	7%

Table V. Net event rates at one year for nulliparous women

Rates	*TCu 300*	*TCu 220C*
Event		
Pregnancy	0.9 (0.2-2.6)	0.6 (0.1-2.2)
Expulsions	3.1 (1.3-5.0)	4.0 (1.8-6.2)
Removals		
Pain/bleeding	9.6 (6.4-12.8)	12.7 (9.0-16.4)
Other medical	2.4 (1.1-4.7)	0.9 (0.2-2.7)
Planned pregnancy	2.4 (1.1-4.7)	2.8 (1.3-5.3)
Other personal	2.3 (1.0-4.6)	2.2 (0.9-4.5)
All causes	20.8 (16.4-25.2)	23.3 (18.5-28.0)
Continuation	79.2	76.7
Number enrolled	363	337
Woman months	3,650	3,397
Lost to follow-up	8%	5%

Table VI. Net event rates at one year for multiparous women

Rates	*TCu 300*	*TCu 220C*
Event		
Pregnancy	1.4 (0.2-5.2)	1.5 (0.2-5.3)
Expulsions	2.8 (0.8-7.2)	3.0 (0.8-7.5)
Removals		
Pain/bleeding	8.6 (3.8-13.3)	18.4 (11.8-25.1)
Other medical	1.4 (0.2-5.1)	0.7 (0.02-4.0)
Planned pregnancy	3.6 (1.3-8.2)	3.7 (1.2-8.7)
Other personal	1.4 (0.2-5.1)	2.3 (0.5-6.7)
All causes	19.2 (12.6-25.9)	29.6 (21.7-37.4)
Continuation	80.8	70.4
Number enrolled	153	146
Woman months	1,556	1,348
Lost to follow-up	10%	10%

tolerated by nulliparous women.[8] (2) There is less menstrual blood loss in both nulliparous and multiparous women with the copper devices.[9] The major disadvantage has been the need for periodic replacement, which is not necessary for the plastic devices. The results of this randomized study indicate that the TCu 220C, which is not expected to have this disadvantage, has event rates similar to those of the wire-wound TCu 300 in the first year of use. If long-term follow-up confirms these findings, the TCu 220C could become the IUD of choice for all women.

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Effect of clomiphene citrate on serum prolactin in infertile women with ovarian dysfunction

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Serum prolactin (PRL) levels were determined by radioimmunoassay in 12 normal, menstruating women and in 26 infertile women with ovarian dysfunction for one cycle or for about 30 days in amenorrheic women. Galactorrhea was not observed. No significant change in serum PRL levels was observed throughout the normal, menstrual cycle. Of the 26 patients with ovarian dysfunction, seven showed PRL levels higher than normal. Twelve of the 26 patients were treated with clomiphene citrate (Clomid†), 100 mg. daily for 5 days. Ovulation occurred in seven, and pregnancy was achieved in two of them. Serum PRL, follicle-stimulating hormone, and luteinizing hormone were measured for a preceding control cycle and during the cycle following Clomid treatment in the 12 patients. The serum PRL levels were normal during the control cycle in five of the patients who ovulated with Clomid and high in four patients who failed to ovulate. Although serum PRL levels were not significantly changed by Clomid in the patients who ovulated with the drug, they were markedly decreased during and immediately after Clomid treatment in patients who failed to ovulate with Clomid.

THE MOST OBVIOUS aspect of prolactin (PRL) action in vertebrates is related to reproduction.[1] However, the role of PRL in human reproduction is not certain. Several investigators[2–5] have measured serum PRL during menstrual cycles with a recently developed radioimmunoassay for human PRL and found no consistent pattern. Further, serum PRL levels in patients with ovarian dysfunction have not yet been characterized, though high serum PRL levels were found in women with amenorrhea-galactorrhea syndromes.[6, 7] Therefore, it was our aim to study the variations in serum levels of PRL over a prolonged period of time for two purposes: (1) to compare serum PRL levels in ovarian dysfunction with those of normal, menstruating women; (2) to determine the effect of clomiphene citrate (Clomid) on serum PRL levels in women with ovarian dysfunction.

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Material and methods

Serum PRL levels were determined for one menstrual cycle in 12 normal menstruating women, 19 to 23 years of age. Serum PRL levels were also determined in 26 women with ovarian dysfunction for one cycle or for about 30 days in an amenorrheic patient. The clinical diagnoses were as follows: one, primary amenorrhea; seven, secondary amenorrhea; six, oligomenorrhea; six, anovulatory cycle; and six, short luteal phase. Of the 26 patients with ovarian dysfunction, 12 (one, primary amenorrhea; four, secondary amenorrhea; three, oligomenorrhea; three,

Table I. Mean levels of serum prolactin in patients with ovarian dysfunction

Type of ovarian dysfunction	*Means* ± standard errors*
Primary amenorrhea	47.3 ± 5.6
Secondary amenorrhea	92.4 ± 11.1, 50.9 ± 6.5, 34.7 ± 4.8, 23.3 ± 2.8, 23.0 ± 3.1, 21.5 ± 1.7, 8.2 ± 1.7
Oligomenorrhea	59.2 ± 7.6, 44.5 ± 7.5, 23.5 ± 1.9, 23.0 ± 2.2, 21.4 ± 3.0, 12.1 ± 2.1
Anovulatory cycle	68.3 ± 6.5, 50.3 ± 5.4, 25.4 ± 2.4, 24.6 ± 4.4, 22.7 ± 1.5, 21.1 ± 3.2
Short luteal phase	31.6 ± 7.1, 31.2 ± 2.8, 23.7 ± 2.0, 20.6 ± 2.6, 19.6 ± 2.2, 14.3 ± 1.0

*Serum prolactin levels were expressed in terms of nanograms of HPr V-L-S No. 1 per milliliter of serum.

Table II. Mean levels of serum prolactin in control cycle and in cycle after Clomid treatment

Patient	*Control cycle (A)*	*Cycle after Clomid treatment (B)*	*B/A*	*Difference* between A and B*
Patients who ovulated with Clomid				
N. K.	23.5 ± 1.9†	27.7 ± 2.6	1.17	N.S.
T. K.	50.3 ± 5.4	43.2 ± 7.0	0.86	N.S.
K. K.	25.4 ± 2.4	23.8 ± 1.7	0.94	N.S.
I. K.	13.6 ± 2.2	18.8 ± 2.7	1.38	N.S.
S. S.	23.7 ± 2.0	27.1 ± 1.2	1.14	N.S.
Mean (a)	26.9 ± 2.2	28.1 ± 2.0	1.04	N.S.
Patients who failed to ovulate with Clomid				
A. T.	59.2 ± 7.6	38.3 ± 6.1	0.65	N.S.
S. K.	47.3 ± 5.6	35.5 ± 4.4	0.75	N.S.
Y. S.	23.0 ± 3.1	24.3 ± 2.6	1.06	N.S.
I. M.	50.9 ± 6.5	29.4 ± 2.4	0.58	$p < 0.01$
M. K.	68.3 ± 6.5	51.3 ± 7.2	0.75	N.S.
Mean (b)	52.0 ± 3.4	35.7 ± 2.4	0.69	$p < 0.001$
b/a	1.93	1.27		
Difference* between a and b	$p < 0.001$	N.S.		

N.S. = Not significant.
*Significance of differences between means was determined by the Student's t test.
†Serum prolactin levels were expressed in terms of nanograms of HPr V-L-S No. 1 per milliliter of serum.

anovulatory cycle; and one, short luteal phase) were treated with Clomid in a dose of 100 mg. daily for 5 days from the fifth day of menses or bleeding induced by subcutaneous injection with 50 mg. of progesterone (Proluton, Schering A.G.). The short luteal phase was defined only by a period of eight days or less from the nadir on the basal body temperature (BBT) chart to the onset of menstrual flow. Sera were obtained serially from the 12 patients through two consecutive cycles (one control and one after Clomid treatment). Ovulation was judged by BBT, urinary pregnanediol excretion, endometrial findings, cervical mucus, vaginal smear, and pregnancy. None of the patients studied showed galactorrhea.

Serum PRL levels were determined by radioimmunoassay[8] with the double-antibody solid-phase method[9] for separating antibody-bound from free labeled hormone with slight modifications. Serum PRL concentrations were expressed as nanograms of HPr V-L-S No. 1 per milliliter of serum. The intra- and inter-assay coefficients of variation were 12 and 20 per cent, respectively. Serum levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were measured by radioimmunoassays as previously described[10] and expressed as micrograms of LER 907 per 100 ml. of serum. The intra- and inter-assay coefficients of variation in the radioimmunoassay for FSH were 8 and 10 per cent, respectively, and those for LH were 7 and 14 per cent, respectively. The radioimmunoassay kit for PRL as well as the kits for FSH, LH, HPr V-L-S No. 1, and LER 907 were supplied by the National Pituitary Agency, National Institute of Arthritis, Metabolic and Digestive Diseases, National Institutes of Health.

Results

Serum PRL concentrations during the menstrual cycle. There was no significant difference between serum PRL levels during the follicular phase (17.8 ± 1.3 ng. per milliliter) and those during the luteal phase (17.9 ± 1.4 ng. per milliliter). The mean serum concentration during the menstrual cycle was 17.8 ± 1.0 (standard error) ng. per milliliter.

Serum PRL levels in patients with ovarian dysfunc-

tion. The means (±standard errors) of serum PRL concentrations in the 26 patients with ovarian dysfunction are shown in Table I. Only one patient with primary amenorrhea was studied, and she had high serum PRL levels. The mean serum PRL levels of seven patients with secondary amenorrhea ranged from normal (8.2 ng. per milliliter) to high (92.4 ng. per milliliter). Five of the seven patients had serum PRL levles within the normal range, and the other two had high levels. The serum PRL levels of patients with oligomenorrhea also ranged from normal (12.1 ng. per milliliter) to high (59.2 ng. per milliliter). Of six patients with anovulatory cycles, four had normal serum PRL concentrations and two had high concentrations. All the patients with short luteal phases had normal serum PRL levels.

Effect of Clomid on serum PRL levels in women with ovarian dysfunction. Twelve patients with ovarian dysfunction were treated with Clomid. Ovulation was induced in seven (two, secondary amenorrhea; one, oligomenorrhea; three, anovulatory cycles; one, short luteal phase), and pregnancy was achieved in two patients with secondary amenorrhea.

Comment

This is the first report describing serum PRL concentrations in women with various types of ovarian dysfunction. Although patients with short luteal phases showed serum PRL concentrations within the normal range, patients with amenorrhea, oligomenorrhea, and anovulatory cycles showed serum PRL concentrations which ranged from normal to high. Thus, the present study indicates that patients with ovarian dysfunction may have associated normal or high serum PRL levels. The high serum PRL levels found in some of the patients studied suggest the associated disturbance of regulation of PRL secretion in addition to that of gonadotropins in these patients without galactorrhea.

Of the 12 Clomid-treated patients, seven ovulated. Two of the patients who ovulated showed high serum PRL concentrations, and the other five patients showed normal levels. Of the five patients who failed to ovulate with Clomid, four showed high serum PRL concentrations. These results would seem to indicate that patients with high serum PRL concentrations are not likely to respond to Clomid treatment. However, the findings that two patients with high serum PRL concentrations ovulated with Clomid and that one of them became pregnant contradict this assumption.

Serum PRL concentrations during the cycle with Clomid treatment in the patients who failed to ovulate were lower than those during the control cycle with the exception of one patient in whom serum PRL levels were within normal range (Table II). Clomid appears to lower serum PRL levels in patients whose serum PRL levels are high. Robyn and associates[11] also reported that Clomid administration resulted in lowering of serum PRL levels in most cases where ovulation was induced by Clomid. However, serum PRL levels did not decrease with Clomid in patients with normal serum PRL levels in the present study. The mechanism of action of Clomid on PRL secretion remains unclear at this time. Estrogen promotes PRL secretion in rats[12] and in hypogonadal women.[13] Accordingly, Clomid might lower serum PRL levels by its established antiestrogenic effect.[14] Amenorrhea-galactorrhea syndromes are characterized by high circulating PRL levels and low or normal excretion of total urinary gonadotropin[15] or low or normal circulating gonadotropins.[7, 16–18] In such patients, a decrease in serum PRL induced with 2-Br-α-ergocryptine (CB-154)[7, 15] or L-dopa[17] was followed by rises in gonadotropin secretion and ovulation. Precipitous fall in serum PRL induced by CB-154 also was associated with a significant rise of serum FSH in normal postpartum women as in the case of amenorrhea-galactorrhea syndrome.[19] The decrease in serum PRL appears to stimulate gonadotropin secretion in these patients. However, two patients with high serum PRL levels ovulated in the present study. Ehara and colleagues[5] described ovulation observed in women in whom serum PRL was increased with amphetamine. Recently, Zarate and co-workers[20] reported that the serum PRL increase induced by thyrotropin-releasing hormone did not interfere with ovulation in women. Thus, the high circulating PRL levels may inhibit gonadotropin secretion in women with the amenorrhea-galactorrhea syndrome, but high serum PRL levels are compatible with ovulation in some cases of ovarian dysfunction without galactorrhea. The relationship between PRL and gonadotropin secretion appears to be complicated and individualized in various cases of ovarian dysfunction.

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CO_2 hysteroscopy: Diagnosis and treatment

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The authors have been using hysteroscopy for intrauterine examination and also for transuterine tubal sterlization for some years, and have adopted the use of CO_2 gas as the distension medium. They explain their suggested values, review their experience, and state their reasons for believing that hysteroscopy is safe and should be more widely used.

HYSTEROSCOPY is one of the oldest endoscopic examination methods used in gynecology; it is also called utero- or metroscopy. Pantaleoni[20] introduced the first hysteroscope in 1869. Recent improvements in optical and technical instruments have renewed interest in this procedure. For the past 3½ years we have been interested in the use of hysteroscopy to detect intrauterine diseases and subsequently for transuterine tubal sterilization. We have tried all of the known fluids as distension media. Based on our experiences with laparoscopy and tubal pertubation, we adopted the use of CO_2 gas as the distension medium to produce a permanent pneumometra. Compared with other techniques, we think this is the most suitable way to distend the uterine cavity—which is, under normal conditions, a shallow cleft.

In 1970 we started performing the examinations with a 10 Ch. endoscope, but we found that the information obtained of the uterine cavity with the use of a 2.7 mm. optic was insufficient and the light was too weak. For the last 2½ years we have worked with a hysteroscope 7 mm. in diameter. A foroblique optic of 4 mm. enlarges the cavity much better and in addition the light quality is improved. This instrument, made by Storz,* can also be used for the cystoscopy. Through a working channel one can insert a urinary catheter for tubal sounding, an electroprobe for cauterization, and a biopsy forceps or other instruments. In order to prevent the escape of CO_2 gas we developed a hysteroscope adaptor* which is necessary to maintain a permanent pneumometra. For gas insufflation we have used in the last year only the Hysteroflator 1000,† a CO_2 insufflation apparatus made expecially for hysteroscopy. By means of its reliably adjusted technique, the desired quantity of gas for insufflation can be predetermined. An electronic CO_2 gas-volume gauge monitors the insufflated gas. A constant insufflation pressure guarantees a continuous gas flow and consequently a pneumometra for a sufficiently distended uterine cavity. Manometers of the apparatus show exactly the gas flow rate, that is, the gas quantity per minute which is insufflated into the uterus and the intrauterine pressure. The principle of the Hysteroflator is as follows: If the intrauterine pressure increases, the flow drops; the flow stops when a pressure of 200 mm. Hg is achieved. So no complications can result from too much insufflated gas or too high an intrauterine pressure.

The procedure is performed in the proliferative phase, because one obtains at this time the best visualization of the uterine cavity. The endometrium is not abundant and in general the tendency to bleeding and the mucus production is minimized. After careful cervical dilatation to Hegar 7 a hysteroscope is inserted, the hysteroscope adaptor fixed by vacuum on the cervix, and the pneumometra produced. Normally, for permanent distension of the uterine cavity, we used a gas flow of 40 to 60 ml. per minute, while the intrauterine pressure ranges between 40 and 80 mm. Hg. The procedure was originally performed under general anesthesia but in the last two years we have used only local anesthesia. We produce a paracervical

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Table I. Indications and contraindications for hysteroscopy

Indications:
1. Premenopausal bleeding
2. Abnormal bleeding
3. Infertility and sterility
   (a) Habitual abortion
   (b) Incompetent cervix
   (c) Enlarged postcoital test
4. Sterilization
5. Deformations and abnormalities of the uterus
6. Evaluation and staging of carcinoma (corpora)
7. Lost IUD

Contraindications:
1. Gravidity
2. Incomplete abortion
3. Heavy bleeding
4. Severe purulent endometritis
5. Infected pelvic inflammatory disease

Table II. Abnormalities found in 1,100 diagnostic hysteroscopies

	No.
Corpus carcinoma	6
Uterus arcuatus	8
Uterus unicollis bicornis	3
Uterus subseptus	14
Synechias	42
Submucous fibromas	58
Polyps	91
Polypous endometrium	96
Total	318
	28.9%

Table III. Abnormalities found in 145 infertility and sterility patients

	No.
Polyps	25
Polypous endometrium	27
Fibromas	9
Synechias	16
Uterus septus	4
Uterus arcuatus	4
Total	85
	58%

block with 1 per cent Xylonest,* 7 ml. for each side. We have not observed any side effects and analgesia is almost immediate.

After reviewing more than 1,695 hysteroscopies we have found the indications and contraindications for this procedures listed in Table I.

Visualization of the uterine cavity for diagnostic evaluations and therapeutic procedures was essentially satisfactory in nearly all 1,695 hysteroscopies. One is able to identify details of the cavity and to distinguish between typical pictures of the endometrium according to the phase of the cycle at the time of inspection. Further, one can recognize small, flat, embedded, submucous fibromas, synechiae, mucous membranes, septa, polyps, and carcinomas which are without clinical symptoms and which cannot be discovered by dilatation and curettage or hysterosalpingography because of their minute sizes. The combination of hysteroscopy and exploratory laparoscopy seems especially a progressive result in discovering the causes of sterility and infertility. It is possible to obtain information about intrauterine diseases, the patency of the tubes by chromopertubation, and the interrelationship of the pelvic organs.

The raw data of the diagnostic evaluations are summarized in Table II. A total of 1,100 patients have been examined for diagnostic reasons. In 28.9 per cent we observed pathologic anatomical changes of the endometrium. Mainly we found a polyposis in 96 cases which means histologically glandular cystic hyperplasia of the endometrium; 91 patients had polyps; submucous fibroids were found in 58 cases, uterine malformations in 17 cases, and in three a corpus carcinoma could be diagnosed without clinical symptoms.

Hysteroscopy was performed on 145 infertility and sterility patients. Table III shows the results. In 58 per cent we recognized pathologic changes of the uterine cavity as malformations,[8], synechiae,[16] polypous endometrium,[27] fibromas,[9] and polyps.[25]

Therapy

For 2½ years we have been accumulating data and experiences with transuterine electrocoagulation of the pars intramuralis tubae by means of the hysteroscope as a method for sterilization. Up to now we have treated more than 450 women. Follow-up examinations of 267 cases after about 12 weeks and later showed a success rate of 90.2 per cent, if we coagulated the patients in the early proliferative phase of the cycle with a low temperature of about 90° C. for 60 seconds. For electrocoagulation we employ a high-frequency apparatus of Siemens,* Radiotom 617, and a 60 cm. long electroprobe by Lindemann of 1 mm. diameter. The probe is equipped with an insulation cap to prevent frontal warmth generation, so that thermal injuries to the outer wall or the neighboring organs cannot lead to complications. The heat generated develops nearly radially and can reach only the myometrium.

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Table IV. Summary of 267 follow-up examinations 3 months after transuterine tubal sterilization

Phase of the cycle	*No. of cases*	*Occlusion*	*Patency*		*Gravidity*
			One	*Both*	
Patients, 242; Temperature, 90° C.; Time, sec.: 60					
Proliferative phase	234	211	14	9	6 intrauterine, 1 ectopic, 1 interstitial, =3,4%
		90.2%	9.8%		
Secretory phase, 4 to 7 days post abortion	8	0	5	3	3
Patients, 33; Temperature, 140° C.; Time, sec.: 5 to 7					
Proliferative phase	23	15	2	6	3
Secretory phase, 4 to 7 days post abortion	10	0	4	5	4

Our conclusions are summarized in Table IV. A total of 234 patients treated in the early proliferative phase, with a coagulation temperature of 90 ° C. and about 60 seconds' duration, were followed up for 3 months and had a hysterosalpingography or a control hysteroscopy. Of these, 211 (90.2 per cent) had bilateral tubal occlusion; 14 of these had unilateral and nine bilateral patency of the tubes. Eight patients in this group became pregnant (six intrauterine, one ectopic, one interstitial). Three pregnancies were observed in patients treated in the secretory phase with 90° C. or 4 to 7 days after termination. Table IV shows the results in 33 patients treated with 140° C. for 5 to 7 seconds. We recognized bilateral tubal occlusion only in 15 cases. The rate of tubal closure was lower than in the other group. There were three pregnancies in this group treated in the proliferative phase and four treated 4 to 7 days after termination became pregnant. We found that the procedure performed in the early proliferative phase with low temperature (90° C.) and the time of about 60 seconds resulted in a higher bilateral occlusion compared with only 65 per cent of the other group.

The pregnancy rate in our selected material is 3.4 per cent (six intrauterine, one ectopic, one interstitial). We observed four pregnancies in a group of patients who were in the first 3 months after hysteroscopic sterilization and were not using contraceptives. We cannot confirm the theory of functional sterility (Mikulicz-Radecki, 1926; Kipfer, 1950; Kneer, 1951; Horstmann, 1952, Moricard, 1956; Black and Asdell, 1958; Rauscher, 1969; Stegner, 1969; Koster, 1970). This theory states that sterility could occur although the tubes remain open, as long as their musculature and the "sphincter mechanism" have been destroyed. We are unable to prove this theory wrong or right at the moment, because it is impossible to examine the treated tubes histologically in order to see whether the musculature of the pars intramuralis has been damaged or not. From the practical point of view we only can recommend the use of contraceptives until a definitive tubal occlusion is confirmed. Two pregnancies were observed after occlusion was diagnosed by hysterosalpingography and one pregnancy after tubal insufflation control. It is possible that we opened the occlusion again by the control examinations or a recanalization of the tubes has taken place. Another reason could be a diagnostic failure of the hysterosalpingography—that it was only a tubal spasm and not a real occlusion.

Table V. Complications by 450 transuterine tubal sterilizations

	No.
Pelveoperitonitis	2
Perforation with electroprobe	1
Total	3

Complications

We did not observe any complications in the group of more than 1,245 diagnostic hysteroscopies. Two perforations were recognized in the group of 450 transuterine tubal sterilizations (Table V). In one of these cases the coagulation caused a necrosis of the bowel; in the other case only the uterine wall was

perforated. These complications could have been avoided by greater caution on the part of the operator. Two patients suffered from slight peritonitis some days after the procedure. Hysteroscopy seems to result in a very low complication rate.

After a review of about 3,200 CO_2 hysteroscopies in Germany we can say that this is a simple and a safe method as long as one adheres to the suggested values for insufflation. Some CO_2 complications have occurred in other hospitals and not with the insufflation apparatus we use.

The complications observed include cardiac arrhythmias and even cardiac arrest, probably due to acidosis. In each case too much gas, up to 1,000 ml. per minute, had been insufflated, and possibly it entered the bloodstream. An inadequate insufflation apparatus was used. All these patients recovered without any serious consequences. To obtain some data about the CO_2 behavior we use we undertook blood-gas measurements (pH, Pco_2, and Po_2) and electrocardiograms (ECG) during 55 hysteroscopies. We extended the observation time to 20 minutes and we found no significant changes in the blood gases of the patients. There was also no difference if the procedure was done under local or general anesthesia. The amount of CO_2 which can be absorbed depends on (1) Pco_2, (2) pH, (3) percentage of oxygen saturation of hemoglobin, (4) temperature, and (5) blood flow per minute.

We tried to determine, in our animal experiments, the compatibility of CO_2 injections and possible complications. In a group of 20 German shepherd dogs different CO_2 amounts were injected for 15 minutes into the vena femoralis under a pressure of 200 mm. Hg. The following measurements were made in all animals: (1) arterial blood pressure; (2) arterial blood pH, Pco_2, Po_2; (3) ECG changes; (4) heart rate; (5) respiratory rate. Clinical symptoms were also observed. We could recognize that up to a flow rate of 200 ml. per minute for 5 minutes no significant changes in blood gas, blood pressure, ECG, or any other symptoms were noticed. After this time changes could be observed. A flow rate of 400 ml. per minute and more caused an increase in the frequency of the heart and deeper respiration. There were also changes in the ECG such as extrasystoles and a depression of the ST-segment. The arterial blood Pco_2 increased, Po_2 decreased, and the pH changed to a slight acidosis. Clinical symptoms such as tachycardia, tachypnia, reduced respiratory rate, and restlessness were found. The blood pressure and pupillary reflex were nearly the same. Increasing the CO_2 up to 1,000 ml. per minute resulted in bradycardia, an increase of the Pco_2, a clearly visible decrease of the Po_2, and a pH change to acidosis. Two minutes after the CO_2 injection the reflexes were extremely diminished and then apnea resulted. The blood pressure dropped from 100 to 50 mm. Hg and even lower. The ECG showed arrhythmias and right bundle branch block.

The threshold dose for toxic reactions is about 400 ml. per minute for dogs. Lethal dosage was between 400 and 1,000 ml. per minute. Death occurred after a latency period of about 100 to 120 seconds. Autopsy performed immediately after death did not show any signs of a CO_2 embolus. The main cause of these symptoms was the acidosis which resulted from the too high gas flow rate (insufflated gas quantity per minute).

To prove the safety of any gas being injected intravenously, it must be demonstrated that the entire amount could be tolerated if shunted from the right to the left heart through a septal defect. Therefore we injected CO_2 directly into the carotid artery in another group of German shepherds dogs. The flow rate was 100 ml. per minute under a pressure of 200 mm Hg. Even in this group no significant clinical symptoms of embolism were detected. We can confirm the experimental work of Oppenheimer and associates,[21] Durant and associates,[5] Stauffer and associates,[27] and Hoeffken and associates,[9] who also did animal experiments to prove the safety of CO_2.

To transfer these observations to human subjects we must compare the minute blood volume of the dogs with that of man to find the limits of compatability. The quality of CO_2 tolerated is directly proportional to the cardiac output. Only when a greater volume of CO_2 gas per minute is used can complications be expected; if appropriate volume and pressure are applied, problems will be avoided.

We assume that CO_2 hysteroscopy using the above mentioned insufflation values is safe. Rubin[23] reported figures collected from 380 authors and found no CO_2 complications among 80,000 tubal pertubations.

Comment

CO_2 hysteroscopy is a simple and safe method which can be performed as an outpatient procedure under local anesthesia. We think that this procedure is out of the experimental stage and will be a good diagnostic and therapeutic tool for the gynecologist. Hysteroscopy is a direct method for the evaluation of intrauterine diseases and is far better than hysterosalpingography and the information obtained only by dilatation and curettage. Englund and associates[7] proved this in very good work. They reported about a 50 per cent failure rate of hysterosalpingography compared with their hysteroscopic results, and they missed many polyps and fibroids by doing only a



dilatation and curettage in comparison to hysteroscopic evaluation. Hysteroscopy is adding more precision to the diagnosis than hysterograms, endometrial biopsy, or curettage, because the uterine lesion can be visualized and a direct biopsy can be obtained.

As CO_2 hysteroscopy enables a good visualization of the uterotubal junction it is easy to perform transuterine tubal sterilization by cauterization of the intramural part of the tubes. The success rate has been about 90 per cent when the patients were treated in the early proliferative phase with a coagulation temperature of 90° C. As there are some failures in the diagnosis of tubal occlusion after transuterine tubal sterilization, we think the best method of evaluating the success of a transuterine sterilization is by hysteroscopy with the Hysteroflator 1000. By this control examination the apparatus indicates a closure by intrauterine pressure of 200 mm. Hg and a flow rate of zero. By hysteroscopy one can observe the former area of the ostium to see if there is scarring and occlusion. On visualization, if some CO_2 bubbles disappear, one can be sure that it still remains open. Another symptom of tubal patency after sterilization could be the shoulder pain which also appears after a Rubin test if the tubes were patent. By this control hysteroscopy we can avoid as far as possible diagnostic failures of tubal occlusion.

Conclusion

More than 3,200 CO_2 hysteroscopies have been performed in Germany without serious complications. We have proved, with our blood-gas analysis and our dog experiments, that CO_2 hysteroscopy is not hazardous. It is absolutely safe if our recommended values are used.

In our opinion CO_2 is the best distension medium for the uterus because it is a gas natural to the body in contradistinction to the other media which are foreign to the body. CO_2 allows the visualization of the tissue and the identification of intrauterine diseases very clearly and the view is true to nature because it has the same optical qualities as air. Finally, from the practical point of view, CO_2 is the best medium for the use and long life of the instruments.

At the present time, when technologic process has taken such a large step forward, and when all medical disciplines wish to further their knowledge by inspecting the various body cavities, the endoscopic observation of the uterus, which especially provides more subtle diagnosis, should find a place as one of the common diagnostic examination methods in our gynecologic practice.

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Diagnostic and therapeutic efficacy of cervical conization

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Cervical conization was performed on 756 patients at the University of Kentucky Medical Center from July 1, 1964, to January 1, 1973. Sixty-six patients were pregnant at the time of conization. Eighty-six per cent of patients with cytologic findings of carcinoma in situ had histologic verification of carcinoma in situ or severe dysplasia, and there was absolute correlation between cytology and histology in 75 per cent of patients with occult invasive cancer. Cervical biopsies without colposcopic direction predicted either severe dysplasia or carcinoma in situ in 77 per cent of cases but were accurate in only seven of 24 patients with occult invasive cancer. Carcinoma in situ was present in 30 per cent of hysterectomy specimens following conization but recurred in only seven per cent of patients followed without hysterectomy. Recurrent carcinoma in situ following hysterectomy was more common in patients with residual intraepithelial cancer in the uterus but was independent of the size of the vaginal cuff removed. Major postconization complications requiring hospitalization occurred in 3.4 per cent of nonpregnant patients and in 7.5 per cent of pregnant patients.

SINCE ITS INITIAL description by Lisfranc[1] in 1815, the technique of cervical conization has undergone many changes. Sims,[2] in 1861, closed the cervix with silver wire sutures following conization and was the first to obtain healing by primary intention. Likewise, Sturmdorf,[3] in 1916, described a method inverting cervicovaginal epithelium to cover the conization site which is still used in many institutions today. More recently, several investigators[4, 5] have described methods of injecting vasoconstrictive solutions into the cervix at the time of conization in order to minimize blood loss. Despite these technical advances, cervical conization remains a major operative procedure associated with a five to ten per cent incidence of postoperative complications.[4, 6]

Perhaps even more important than changes in conization technique have been changes in the indications for its performance. Whereas conization was initially performed for invasive cervical cancer and the repair of cervical lacerations,[7] it has been used during the past three decades for the diagnosis and treatment of cervical epithelial abnormalities. With the utilization of colposcopy to direct cervical biopsies, it has been suggested by some investigators that cervical conization is being performed too frequently.[8, 9]

The present study was undertaken to determine the diagnostic and therapeutic efficacy of cervical conization as a method in the evaluation of patients with cervical neoplasia.

Methods and results

From July 1, 1964, to January 1, 1973, cervical conization was performed on 756 patients at the University of Kentucky Medical Center. Sixty-six of these patients were pregnant at the time of conization.

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Conization was carried out as a diagnostic method in patients with cytologic or biopsy findings of cervical epithelial abnormalities. Cytologic and histologic abnormalities were graded according to the following classification: mild dysplasia, moderate dysplasia, severe dysplasia, atypical cellular changes, carcinoma in situ, microinvasive carcinoma, and invasive carcinoma. Cervical conization was performed in the absence of biopsy-proved invasive cancer within two months on all patients with cytologic or biopsy evidence of persistent moderate dysplasia, severe dysplasia, carcinoma in citu, or microinvasive carcinoma. Persistent moderate dysplasia was defined as moderate dysplasia on two occasions separated by at least three months. Microinvasive cancer was defined as the presence of stromal invasion to a depth of less than 5 mm.

Conization was performed according to the method of Parker and colleagues.[10] Twelve to sixteen sections were made of each conization specimen, and additional step sections were cut when an area indicative of epithelial abnormality was noted. Areas of cervical epithelial abnormality were described as involving squamous epithelium, both glandular and squamous epithelium, or glandular epithelium alone.

Basic characteristics of the patients studied are presented in Table I. The mean age, gravidity, and location of cervical epithelial abnormality was essentially the same in those patients who were pregnant and in those who were not.

Seven hundred and seven patients had cervical conizations at this institution within eight weeks of cytologic examination. Conization findings in these patients are presented in Table II. Cytology and histology specimens were reviewed in all cases in which there was not absolute cytology-histology correlation. Eighty-six per cent of patients with cervical cytologic findings of carcinoma in situ had histologic verification of severe dysplasia or intraepithelial carcinoma on conization. Seventeen patients with normal or mildly dysplastic cytologic findings had severe dysplasia or carcinoma in situ on conization. However, no patient whose cervical cytology was normal or showed mild or moderate dysplasia had histologic evidence of invasive cancer in the conization specimen. Twenty-eight patients (four per cent) with no visible cervical lesions had invasive cancer at conization. Twenty-one of these patients had cytologic diagnoses of invasive cancer, and the remainder had cytologic abnormalities ranging from atypical cellular changes to microinvasive cancer. Age as an independent variable had no direct effect on the cytology-histology correlation. Thirty-eight patients (5.4 per cent) had cytologic findings which were not severe enough to warrant conization. However, cervical biopsy abnormalities were more advanced and indicated the need for conization.

Table I. Basic characteristics of patients undergoing cervical conization

Characteristics	*Nonpregnant*	*Pregnant*
No. of patients	690	66
Mean age (yr.)	37.0	33.3
Mean gravidity	4.6	5.2
Location of lesion		
Squamous epithelium alone (%)	62.6	57.8
Squamous and glandular epithelium (%)	34.5	35.9
Glandular epithelium alone (%)	2.9	6.3

Cervical biopsies were performed on 537 patients prior to conization. Biopsies were taken from areas in the cervix which failed to take up Schiller's stain, since colposcopy was not performed at this institution during the time of investigation. Endocervical curettage was carried out only in those patients with visible endocervical lesions. The histologic correlation between punch biopsies and conization is presented in Table III. Twenty-four patients with invasive cancer and no visible cervical lesions had punch biopsies prior to conization. Invasive cancer was confined to the squamous epithelium in eleven cases and involved both squamous and glandular epithelium in three cases. Punch biopsies showed microinvasive or invasive cancer in nine patients (37.5 per cent) and carcinoma in situ in 11 patients (45.8 per cent). Thirteen patients with suspected invasive cancer on punch biopsy had cervical conization in order to determine the extent of invasion. Seven of these patients had invasive cancer and six had intraepithelial or microinvasive cancer.

The relationship between location of the epithelial abnormality and the diagnostic accuracy of cervical biopsy is presented in Tables IV and V. Cervical biopsies showed either severe dysplasia or carcinoma in situ in 145 of 193 patients (75.1 per cent) with carcinoma in situ limited to the squamous epithelium and in 119 of 151 patients (78.7 per cent) whose carcinoma in situ involved both squamous and glandular epithelium. Diagnostic accuracy of punch biopsies was significantly reduced in those lesions confined to the endocervical glands. Eleven patients had either carcinoma in situ or invasive adenocarcinoma of the endocervix, and only six of them (54.5 per cent) were diagnosed accurately on punch biopsy.

Table II. Cytology-histology correlation in patients undergoing cervical conization

Cervical cytologic findings	No. of patients	Conization findings: Mild dysplasia or less		Moderate dysplasia		Severe dysplasia		Carcinoma in situ		Micro-invasive carcinoma		Invasive carcinoma	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Normal	9	4	44.4	0		1	11.2	4	44.4	0		0	
Mild dysplasia	29	11	37.9	6	20.7	2	6.9	10	34.5	0		0	
Moderate dysplasia	103	9	8.6	32	30.8	9	8.6	49	47.1	4	3.9	0	
Severe dysplasia	163	13	8.0	25	25.3	36	22.1	76	46.6	12	7.4	1	0.6
Atypical cells	19	3	15.8	2	10.5	2	10.5	11	57.9	0		1	5.3
Carcinoma in situ	345	12	3.5	6	1.7	22	6.4	274	79.4	28	8.1	3	0.9
Microinvasive carcinoma	7	0		0		0		4	57.1	1	14.3	2	28.6
Invasive carcinoma	32	0		0		0		7	21.9	4	12.5	21	65.6

Table III. Biopsy-conization correlation in patients with cervical epithelial abnormalities

Biopsy findings	Conization findings: Mild dysplasia or less		Moderate dysplasia		Severe dysplasia		Carcinoma in situ		Micro-invasive carcinoma		Invasive carcinoma	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Normal	3	13.6	12	20.0	6	10.2	27	8.1	2	5.3	0	
Atypical cellular changes	0		1	1.7	1	1.7	6	1.8	0		0	
Mild dysplasia	8	36.4	10	16.7	6	10.2	8	2.4	1	2.6	0	
Moderate dysplasia	5	22.7	23	38.2	15	25.4	32	16.5	3	7.9	3	12.5
Severe dysplasia	4	18.2	13	21.7	19	32.2	55	16.5	3	7.9	1	4.2
Carcinoma in situ	2	9.1	1	1.7	12	20.3	198	59.4	25	65.8	11	45.8
Microinvasive carcinoma	0		0		0		3	0.9	3	7.9	2	8.3
Invasive carcinoma	0		0		0		5	1.5	1	2.6	7	29.2
No. of patients	22		60		59		334		38		24	

Table IV. Punch biopsy–conization correlation according to location of lesion in patients with carcinoma in situ

Location of carcinoma in situ on conization	Patients	Biopsy findings: Severe dysplasia		Carcinoma in situ		Other	
		No.	%	No.	%	No.	%
Squamous epithelium only	193	34	17.6	111	57.5	48	24.9
Squamous and glandular epithelium	151	21	13.8	98	64.9	32	21.3
Glandular epithelium only	1	0		1	100	0	

Sixty-six patients were pregnant at the time of conization and sixty-three of these patients had conization within six weeks of cytologic examination. Thirty-seven (82.2 per cent) of the 45 patients with carcinoma in situ on conization had cytologic evidence of severe dysplasia or carcinoma in situ. The one pregnant patient with occult invasive cervical cancer had cytologic evidence of malignancy. Fifty-three of these patients had cervical biopsies prior to conization. Cervical biopsies were accurate in 78.9 per cent of pregnant patients with carcinoma in situ. However, only one of the three patients with microinvasive cancer was diagnosed on cervical biopsy. The one pregnant patient with invasive cancer was not biopsied

Table V. Punch biopsy–conization correlation according to location of lesion in patients with occult invasive cervical cancer

Location of invasive carcinoma on conization	*No. of patients*	*Biopsy findings*							
		Carcinoma in situ		*Microinvasive carcinoma*		*Invasive carcinoma*		*Other*	
		No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
Squamous epithelium only	11	6	54.5	1	9.1	2	18.2	2	18.2
Squamous and glandular epithelium	3	1	33.3	1	33.3	0		1	33.3
Glandular epithelium only	10	4	40.0	0		5	50.0	1	10.0

prior to conization. Hysterectomy was performed with cesarean section or in the immediate postpartum period in 43 patients with carcinoma in situ. Twelve patients (27.9 per cent) had residiual carcinoma in situ and one had microinvasive carcinoma in the hysterectomy specimen.

Complications of conization are presented in Table VI. Sixteen patients required readmission for excessive cervical bleeding. This bleeding was successfully treated with resuture, vaginal packing, or cryotherapy in thirteen patients, and emergency hysterectomy was required in three patients. Bleeding occurred five to ten days after conization in all patients. Six patients required blood transfusions following conization related bleeding. Uterine perforation at the time of conization occurred in two patients, and three patients experienced spontaneous abortion within one week of conization. The incidence of major conization complications, excluding the 161 patients whose hysterectomy immediately followed frozen section examination of the cone specimen, was 3.4 per cent in nonpregnant patients and 7.5 per cent in pregnant patients.

Three hundred and seventy four patients with carcinoma in situ or microinvasive carcinoma who desired no further pregnancies underwent hysterectomy following conization. Vaginal hysterectomy was performed in 299 patients and abdominal hysterectomy in the remainder. A wide vaginal cuff was excised with the hysterectomy specimen in 226 cases. All hysterectomy specimens were examined microscopically to determine the incidence of residual epithelial abnormality. Sections were routinely taken through the anterior, posterior, and lateral walls of the cervix as well as additional sections in areas of histologic abnormality. No patient had undiagnosed invasive cancer in the hysterectomy specimens following conization. Carcinoma in situ was present in 112 hysterectomy specimens (29.9 per cent) and microinvasive cancer in six (1.6 per cent). Following conization, patients were seen in the gynecologic oncology clinic every three months for two years and every six months thereafter. The duration of follow-up ranged from 12 to 110 months (mean 50 months). Recurrent vaginal cancer following hysterectomy occurred in five patients (1.1 per cent), and there was residual carcinoma in situ in the hysterectomy specimen of four of these patients. Vaginectomy was performed on three of these patients and radiation therapy in two. All of these patients are alive with no evidence of recurrent disease three to 60 months following therapy. One hundred and nine patients with histologically proved carcinoma in situ who desired further pregnancies have been followed without hysterectomy. Seven of these patients (6.4 per cent) have required a second conization and hysterectomy for recurrent carcinoma in situ.

Table VI. Complications of cervical conization

Complications	*Nonpregnant*		*Pregnant*	
	No.	*%*	*No.*	*%*
No. of patients	529		66	
Complication				
Cervical bleeding requiring hospitalization	14	2.6	2	3.0
Uterine perforation	2	0.4	0	
Pelvic infection	2	0.4	0	
Pregnancy loss	0		3	4.5
Total	18	3.4	5	7.5

Comment

The findings of this study indicate that cervical conization is an effective method in the diagnosis of cervical epithelial abnormalities. However, it is a major operative procedure associated with a significant incidence of complications. For this reason, conization should be performed only in those instances in which it

can provide definite diagnostic or therapeutic advantages to less radical procedures. Cervical cytologic examination is an excellent screening procedure and suggests the need for further histologic confirmation by biopsy or conization. Furthermore, cytologic examination and cervical biopsies are often complementary in the diagnosis of cervical epithelial abnormalities. This is exemplified by those patients (5.4 per cent) from this institution with normal or mildly dysplastic cytologic findings whose conizations were performed because of biopsy evidence of more advanced epithelial abnormalities.

The most effective method for reducing the number of cervical conizations is to increase the accuracy of cervical biopsies. In the present series, only seven of 24 patients with invasive carcinoma in the absence of a cervical lesion had the correct diagnosis made by cervical biopsy. Similarly, only 76.8 per cent of the carcinoma in situ or severe dysplasia was diagnosed by biopsy alone. Stafl and Mattingly[11] reported colposcopic prediction of histopathology to be clinically accurate in 85 per cent of patients with cervical epithelial abnormalities. Likewise, Townsend and associates[12] noted an absolute correlation between colposcopically directed biopsies and cervical conization of 84.5 per cent when the lesion did not involve the endocervical canal. Clearly, the accuracy of colposcopy depends upon the skill and experience of the colposcopist. However, it does provide an additional diagnostic alternative to conization if the lesion can be completely defined and the squamocolumnar junction fully visualized.

Conization during pregnancy was directly responsible for a five per cent incidence of fetal loss. In addition, two patients experienced significant postconization hemorrhage. Ortiz and Newton,[13] in a study of 47 pregnant patients with abnormal cervical cytologic findings, found that colposcopically directed biopsy was an effective means to histologically evaluate the cervix and was unlikely to miss invasive cancer because of the physiologic eversion of the cervix during pregnancy. Similarly, Stafl and Mattingly[11] found that no unsatisfactory or incomplete colposcopic examinations occurred in 89 pregnant patients, and conization was not required in any case. Conization during pregnancy is therefore most clearly indicated when the squamocolumnar junction cannot be visualized or when there is biopsy evidence of microinvasive cancer.

The observation that the recurrence rate of carcinoma in situ following conization is significantly less than the incidence of residual intraepithelial carcinoma in the hysterectomy specimen is not new. Creasman and Rutledge[14], in a study of 861 patients treated for carcinoma in situ at the M. D. Anderson Hospital, noted residual carcinoma in situ in 22 per cent of hysterectomy specimens, whereas recurrent carcinoma in situ following conization was found in only seven per cent. In a previous investigation from this institution, van Nagell and colleagues[15] found that the incidence of residual carcinoma in situ following conization was inversely related to the amount of inflammation present in the hysterectomy specimen. Perhaps the inflammatory response of the cervix following conization destroys residual carcinoma in situ or makes epithelial abnormalities difficult to interpret. Recurrent vaginal carcinoma in situ following hysterectomy occurred most commonly in those patients who had residual carcinoma in situ in the hysterectomy specimen but was independent of the size of the vaginal cuff excised.

There is little doubt that the definitive therapy for cervical intraepithelial carcinoma in the woman who has completed her family is hysterectomy. However, for the patient who desires further pregnancy, directed biopsy and conization provide satisfactory diagnostic and, in certain cases, therapeutic alternatives to more radical procedures. In this regard, conization is particularly useful in the diagnosis of lesions extending up the endocervical canal in which the squamocolumnar junction cannot be visualized.

Many advances in the management of malignant cervical disease have come about through greater individualization of therapy. It is not unreasonable to suspect that similar advantages will result from the application of this philosophy to patients with premalignant disease of the cervix.

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Endometrial adenocarcinoma and the polycystic ovary syndrome

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While endometrial adenocarcinoma is the second most common female genital malignancy, only four per cent of the cases occur in women less than 40 years of age. The relative rarity of this disease in young women requires that we be especially attuned to those who are at high risk. This paper presents two case reports of women with polycystic ovarian disease who developed endometrial adenocarcinoma at 24 years of age. The pathophysiology of polycystic ovarian disease and its relationship to the development of endometrial adenocarcinoma are discussed.

ENDOMETRIAL adenocarcinoma, the second most common female genital malignancy, is primarily a disease of older women, with 75 per cent of all cases occurring after the age of 50 and only 4 per cent occurring before the age of 40.[1] While the clinical associations of hypertension, diabetes mellitus, obesity, and nulliparity are well established for older patients, a somewhat different profile is apparent for the very young women who develop endometrial adenocarcinoma.

There have been recent reports of patients with Turner's syndrome who developed endometrial adenocarcinoma after long-term treatment with oral diethylstilbestrol[2–5] and the occurrence of endometrial adenocarcinoma in patients with the polycystic ovary syndrome (PCO) has also been reported.[6–8] Our report of two patients with PCO who both developed endometrial adenocarcinoma at 24 years of age will illustrate the profile of the patient who is at risk for this malignancy at an early age.

Case reports

Case 1. W. S., a 24-year-old, married, black, nulligravid woman, was initially referred because of very heavy "periods" for about 4 months prior to admission. Her menarche had occurred at 15 years of age and menses had always been very irregular. She had also had an increased growth of dark, heavy facial hair for about 6 years.

The patient's height was 65 inches, weight 293 pounds, blood pressure 152/90 mm. Hg. She was extremely obese, with moderate facial hirsutism (Fig. 1). Breast development was good and the findings on pelvic examination were unremarkable, although the examination was somewhat difficult.

Significant laboratory data included: Hemoglobin (Hgb.), 8.0 Gm. per 100 ml.; hematocrit (Hct.), 24 per cent; white blood cell count (WBC), 10,600 per cubic millimeter; platelets, 360,000 per cubic millimeter; serum iron, 55 μg; serum iron-binding capacity, 449 μg per 100 ml.; Hgb. electrophoresis, AA; blood urea nitrogen (BUN), 5 mg. per 100 ml.; bilirubin (total), 0.7 mg. per 100 ml.; glucose (fasting), 90 mg. per 100 ml. Thyroid studies were within normal limits, as were 24 hour urinary 17-ketosteroids and 17-OH steroids. The intravenous pyelogram and skull x-rays were unremarkable and the Papanicolaou smear was negative.

She did not return again for 9 months. During the interval she had had more profuse vaginal bleeding and, at the time of her second admission, the Hgb. was 3.8 Gm. per 100 ml. and Hct. was 15 per cent. After adequate transfusion, a dilatation and curettage was performed and a large amount of tissue obtained which showed both endometrial adenomatous hyperplasia and adenocarcinoma. On obtaining the tissue diagnosis, a total abdominal hysterectomy and bilateral salpingo-oophorectomy were performed. The ovaries were enlarged, with numerous subcapsular follicular cysts but no recent or old corpora lutea. The ovaries showed "capsular" thickening as well as thecal hyperplasia.

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Case 2. S. S., a 24-year-old, unmarried, white, nulligravid woman, had had her menarche at 15 years of age. Her menses had always been very irregular and were usually quite heavy. Ten months prior to admission she had been started on oral contraceptives to "regulate" her menses. Even with oral contraceptives she continued having excessive vaginal bleeding both during and between menses, and just prior to admission a dilatation and curettage was done which showed endometrial adenomatous hyperplasia and adenocarcinoma. She had had moderate facial acne and heavy, dark hair on her chest and breasts for several years.

Her height was 59 inches, weight 170 pounds, and blood pressure 130/-0 mm. Hg. She was moderately obese, with good breast development. She had facial acne and numerous dark hairs on her chest and breasts. Pelvic examination was unremarkable.

Significant laboratory data included: Hgb., 10.3 Gm. per 100 ml.; Hct., 29 per cent; WBC, 4,000 per cubic millimeter; platelets, 286,000 per cubic millimeter; bilirubin (total), 0.6 mg. per 100 ml.; BUN, 10 mg. per 100 ml. Plasma testosterone was 109 ng. per 100 ml. (normal female, 30 to 95). The 17-ketosteroid output (urine) was unremarkable and the Pap smear was reported as negative.

Initial therapy consisted of intrauterine radium, which was followed in one week by total abdominal hysterectomy and bilateral salpingo-oophorectomy. Both ovaries were slightly enlarged with bilateral cortical thickening, numerous follicular cysts, and thecal hyperplasia. No corpora lutea were present but there were several old corpora albicans.

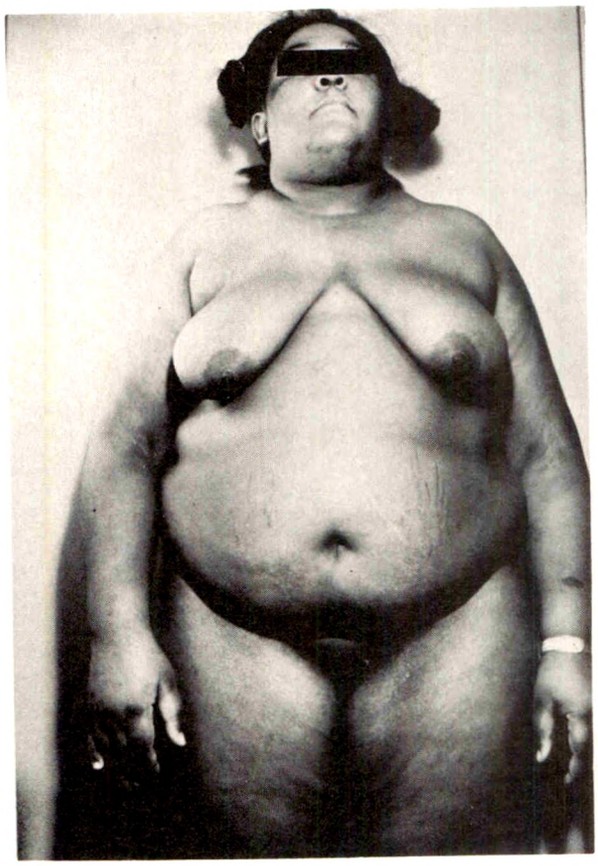


Fig. 1. Patient W. S. (case 1) showing the obesity and hirsutism characteristic of these patients.

Comment

It has only been in the last few years, with the development of sensitive and specific radioimmunoassays for protein and steroid hormones, that the true nature of polycystic ovarian disease has become apparent. Recent work by Yen and associates[9] has shown that women with PCO have chronically depressed serum levels of follicle-stimulating hormone (FSH) as well as chronically elevated levels of luteinizing hormone (LH) which are devoid of any cyclic peaks usually associated with ovulation. The low levels of FSH are consistent with the failure of follicular maturation seen in the PCO ovary and the elevated LH levels explain much of the remainder of the ovarian pathology.

Kirschner and Jacobs,[10] by means of 3 sampling from ovarian and adrenal vein catheters, have shown that the ovary is the major site of androgen production (primarily androstenedione and testosterone) in patients with PCO. Several studies have shown that, in the female, acute treatment with human chorionic gonadotropin (HCG) or LH will result in increased androgen production.[11–13] In view of this growing bulk of data, there can be little question that chronic hyperstimulation of the ovary with LH can result in ovarian hyperthecosis and increased androstenedione and testosterone production. It has also been shown that, in the rhesus monkey, chronic androgen stimulation will result in ovarian capsular fibrosis similar to that seen in the PCO syndrome.[14] It seems, therefore, that the ovarian changes seen in the PCO syndrome are a result of abnormal stimulation rather than being a primary ovarian disorder.

While the cause of the abnormal gonadotropin release is not known, it is known that female rats, if treated with androgens during the first 5 days of life, will later develop a syndrome which is quite similar to the human PCO syndrome, with increased androgen production, polycystic ovaries, and anovulation.[15] It is speculated that this early androgen exposure results in a "masculinization" of the hypothalamus and, while this etiology is unlikely for most human subjects with PCO, the concept of a male-type hypothalamic drive is quite plausible.

The persistent, if asynchronous and incomplete follicular activity in the PCO syndrome does result in persistent estrogen production which may be augmented to a considerable degree by extraovarian conversion of androstenedione to estrone as has been shown by MacDonald and associates.[16] In view of present data, we must assume that this prolonged estrogen stimulation of the endometrium, without the modifying effect of intermittent progesterone, can produce hyperplastic and eve the endometrial glands. The disorder with the progressio neoplastic changes in the end well documented by Chambia

Since the endometrial ade velop in these young women t tiated, the prognosis for s therapy is very good.[18]

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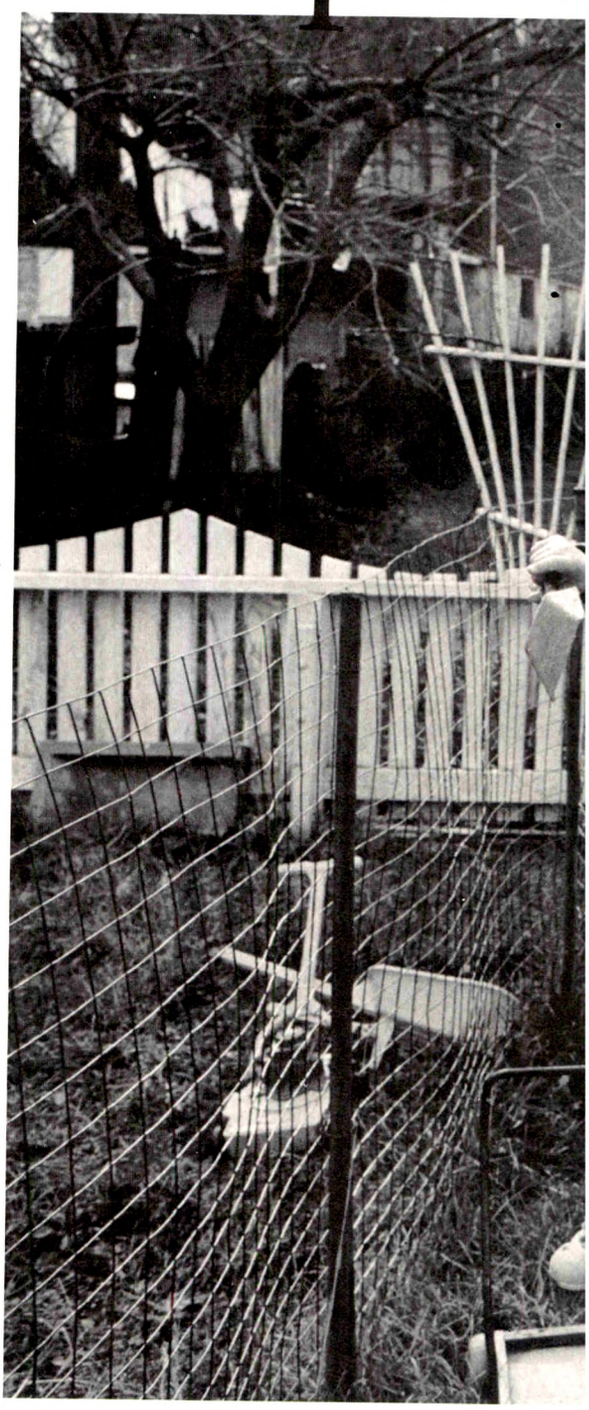
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The woman profiled here is an actual ZORANE 1/50 user. However, not all women can be expected to have the same tolerance for side effects, should any occur. (Data on file, Lederle Laboratories)

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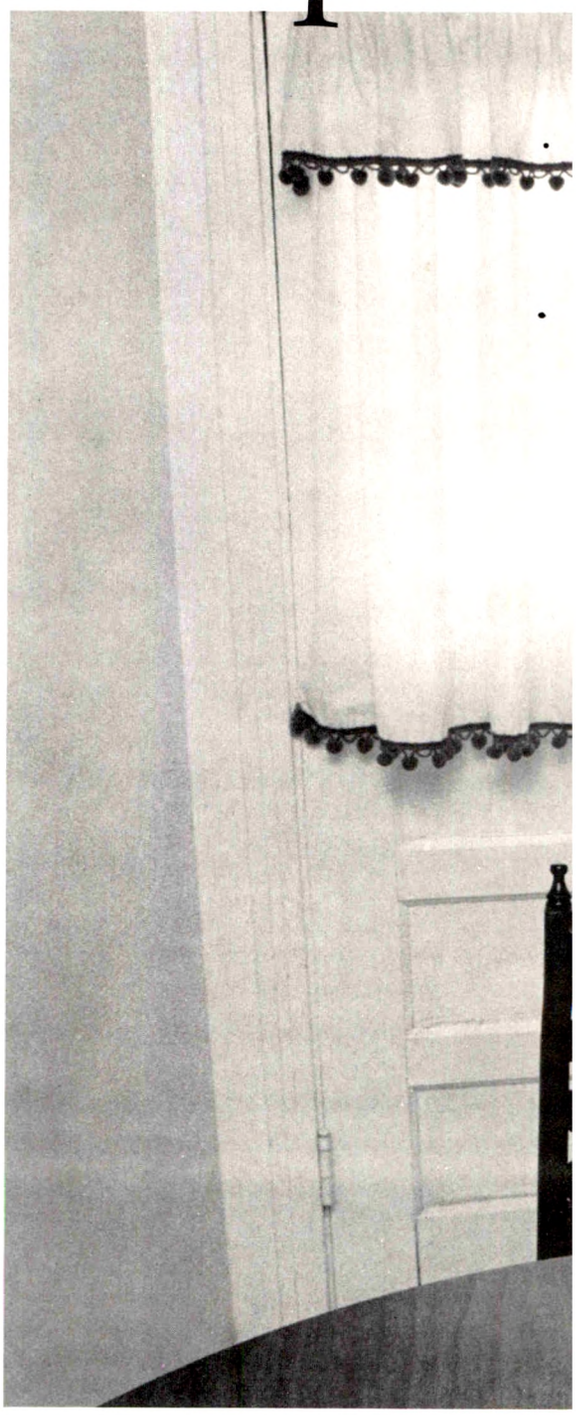
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Zorane® 1/50

Norethindrone Acetate, 1 mg and Ethinyl Estradiol, 50 mcg

Oral Contraceptive Tablets

Progestin/Estrogen content balanced fo

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with



Mrs. Linda McKinnon, Housewife
Nederland, Texas
Age 25, married 7 years, 2 children
Cycle experience with Zorane 1/50: 16

he majority

"You always know when you're going to start... I love knowing that... you can always plan... I think it's great. And, not having any cramps, too, because I used to have cramps."

From real life...

A Woman satisfiec her oral contraceptive



One reason for long-term patient satisfaction: Weight gain usually not a problem

ZORANE 1/50 is a highly effective and generally well-tolerated oral contraceptive—providing the estrogen content and progestin:estrogen ratio that appears to be highly acceptable to the majority of women.

From clinical studies: In a study population of 1156 women, 25,983 cycles, the incidence of weight gain was 0.4%. Actual patient dropout rate* due to weight gain was 2.0% (23 subjects). Overall patient dropout rate was 14.4% due to all adverse reactions. Serious reactions, as well as minor adverse reactions, have been reported following the use of all oral contraceptives. Before, prescribing, the complete product information should be consulted for *special note, contraindications, warnings, precautions* and *adverse reactions*. Thereafter, the physician should remain alert to the earliest manifestations of any serious disorder. Should any occur or be suspected, the oral contraceptive should be immediately discontinued.

The woman profiled here is an actual ZORANE 1/50 user. However, not all women can be expected to have the same tolerance for side effects, should any occur. (Data on file, Lederle Laboratories)

*Dropout rates are based on total patient population (1,156); incidence reflects only those patients who completed one or more cycles (956).

Zorane® 1/50

♀ Norethindrone Acetate, 1 mg and Ethinyl Estradiol, 50 mcg

Oral Contraceptive Tablets

Progestin/Estrogen content balanced fc

See last page of this advertisement for brief summary of prescribing information.

with



Alice Eichenberger, High School
Business Teacher, Louisville, Kentucky
Age 28, married 7 years, no children
Cycle experience with Zorane 1/50: 13

he majority

"I've fluctuated between 115 and 120...and have had no appreciable weight gain...since I've been on Zorane."

Zorane® 1/50

Progestin/Estrogen content balanced for the majority

Norethindrone Acetate, 1 mg and Ethinyl Estradiol, 50 mcg

Oral Contraceptive Tablets

Also available from Lederle

Zorane 1.5/30 Norethindrone Acetate, 1.5 mg. and Ethinyl Estradiol, 30 mcg.

Zorane 1/20 Norethindrone Acetate, 1 mg. and Ethinyl Estradiol, 20 mcg.

Special Note: Oral contraceptives have been marketed in the United States since 1960. Reported pregnancy rates vary from product to product. The effectiveness of sequential products appears to be somewhat lower than that of combination products. Both types provide almost completely effective contraception. The dropout rate for medical reasons, as observed in clinical trials, with ZORANE 1.5/30 appears to be the same as that of higher dose combination products. The dropout rate due to irregular bleeding was somewhat higher, but dropouts were equally split between irregular bleeding and other medical reasons attributable to the drug. (ZORANE 1/20 only: The effectiveness is somewhat lower than that of the higher dose combination products. Both provide almost completely effective contraception. Dropout rate for medical reasons appears to be somewhat higher than with higher dose combination products, due particularly to irregular bleeding.) An increased risk of thromboembolic disease associated with use of hormonal contraceptives has now been shown in studies conducted in both Great Britain and the United States. Other risks, such as those of elevated blood pressure, liver disease, reduced tolerance to carbohydrates, have not been quantitated with precision. Long-term administration of both natural and synthetic estrogens in subprimate animal species in multiples of the human dose increases the frequency for some animal carcinomas. These data cannot be transposed directly to man. Possible carcinogenicity due to the estrogens can neither be affirmed nor refuted at this time. Close clinical surveillance of all women taking oral contraceptives must be continued.

Indications: For control of conception.

Contraindications: Thrombophlebitis, thromboembolic disorders, cerebral apoplexy, or a past history of these conditions; markedly impaired liver function; known or suspected carcinoma of the breast; known or suspected estrogen-dependent neoplasia; undiagnosed abnormal genital bleeding; pregnancy.

Warnings: If early signs of thrombotic disorders (thrombophlebitis, cerebrovascular disorders, pulmonary embolism, retinal thrombosis) occur or are suspected, discontinue use of drug immediately. (Studies indicated a higher risk of these conditions with use of these drugs, not persisting after drug stoppage and not enhanced by long-term use.[1-4]) If sudden partial or complete loss of vision occurs, or if there is sudden onset of proptosis, diplopia or migraine; or if examination reveals papilledema or retinal vascular lesions, discontinue medication. Safety of use in pregnancy has not been established, therefore rule out pregnancy in any patient who has missed two consecutive periods before continuing contraceptive regimen; if prescribed schedule has not been adhered to, consider possibility of pregnancy with first missed period. A small fraction of hormonal agents has been identified in the milk of mothers receiving these drugs; long-range effect to the nursing infant has not yet been determined.

Precautions: Pretreatment and periodic physical examinations of breasts and pelvic organs should include Papanicolaou smear. (Estrogens have produced tumors, some malignant, in 5 species of subprimate animals.) If endocrine and liver function tests are abnormal in patients taking these drugs, repeat tests after drug withdrawal for 2 months. With use of these drugs, pre-existing uterine fibromyomata may increase in size; and some conditions (*i.e.*, epilepsy, migraine, asthma, cardiac or renal dysfunction) may be influenced by fluid retention; observe carefully for such. With use of ZORANE 1.5/30 or ZORANE 1/20, alteration in menstrual patterns may be induced. Since the amount and duration of flow and cycle length may vary, this must be considered in attempting to determine if cause of bleeding is organic. (ZORANE 1/50 only: In breakthrough bleeding or all cases of irregular bleeding per vaginam, keep in mind nonfunctional causes and take adequate diagnostic measures.) With history of psychic depression, discontinue drug if depression recurs to a serious degree. Possible influence of prolonged ZORANE therapy on pituitary, ovarian, adrenal, hepatic or uterine function awaits further study. A decrease in glucose tolerance has been seen in some patients on oral contraceptives; carefully observe diabetics on such therapy. Patient age is no absolute limiting factor, although these drugs may mask onset of climacteric. The pathologist should be advised of ZORANE therapy when relevant specimens are submitted. Susceptible women may have an increase in blood pressure following use of contraceptive steroids.

Adverse Reactions Observed in Patients Receiving Contraceptives: Statistically significant association demonstrated between use of oral contraceptives and: thrombophlebitis, pulmonary embolism, cerebral thrombosis. Suggestive evidence, neither confirmed nor refuted, between use and neuro-ocular lesions (*e.g.*, retinal thrombosis, optic neuritis). Known to occur: nausea, vomiting, G.I. symptoms (abdominal cramps, bloating), breakthrough bleeding, spotting, change in menstrual flow, amenorrhea during and after treatment, edema, chloasma or melasma, breast changes (tenderness, enlargement, secretion), weight change (increase or decrease), changes in cervical erosion and secretions, suppression of lactation when given immediately post-partum, cholestatic jaundice, migraine, allergic rash, rise in blood pressure in susceptible women, mental depression. Reported association with use, neither confirmed nor refuted: anovulation post treatment, premenstrual-like syndrome, changes in libido, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, fatigue, backache, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, itching. *May Alter Laboratory Results in:* hepatic function (increased sulfobromophthalein retention and other tests), coagulation tests (increase in prothrombin, Factors VII, VIII, IX, X), thyroid function (increase in PBI_1, and butanol extractable protein bound iodine, and decrease in T^3 uptake values), metyrapone test, pregnanediol determination.

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OBSTETRICS

The metabolic clearance rate of dehydroisoandrosterone sulfate

IV. Acute effects of induced hypertension, hypotension, and naturesis in normal and hypertensive pregnancies

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In the present study, the metabolic clearance rate of dehydroisoandrosterone sulfate (MCR_{DS}) decreased in normotensive gravidas during short-term studies utilizing angiotensin II–induced elevation of blood pressure. Therapy with hydralazine hydrochloride in chronic hypertensive gravidas resulted in a decrease in blood pressure and an accompanying decrease in the MCR_{DS}. A variable response was observed in the apparent volume of distribution of DS (AVD_{DS}) during therapy with hydralazine hydrochloride. Decreases in MCR_{DS} also occurred in chronic hypertensive gravidas following therapy with 40 mg. of intravenously administered furosemide despite a failure of blood pressure to be altered. The AVD_{DS} increased in four of five patients receiving furosemide, suggesting a possible direct action of furosemide upon vascular smooth muscle.

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IN A PRECEDING report,[10] the effects of chronic administration of thiazide diuretics upon the metabolic clearance rate of dehydroisoandrosterone sulfate (MCR_{DS}) were measured in normal and future preeclamptic patients. In all but one instance, the MCR_{DS} decreased following 1 week of thiazide diuretics.

The purpose of the present study was to measure acute changes in MCR_{DS} evoked by the administration of angiotensin II in normotensive volunteers and following the administration of hydralazine hydrochloride and furosemide to near-term gravidas with

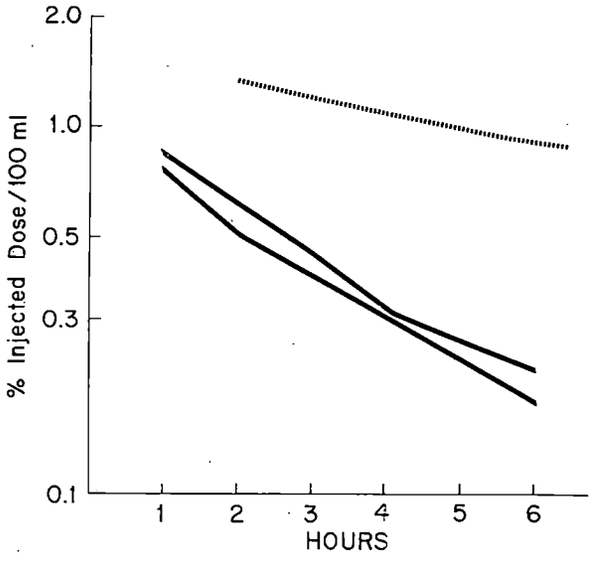


Fig. 1. Disappearance curves of tritium-labeled dehydroisoandrosterone sulfate (^{3}H-DS). The dotted line represents the disappearance of ^{3}H-DS in a nonpregnant subject. The solid lines are those of term pregnant patients. Note that in term pregnant subjects the disappearance rate is increased.

essential hypertension. In all instances, these drugs decreased the MCR_{DS} and in some instances marked decreases occurred which corresponded well with the clinical condition and gross pathologic description of the placenta.

Materials and methods

The MCR_{DS} was measured following the intravenous injection of tritium-labeled DS by determining the concentration of ^{3}H-DS remaining per 100 ml. of plasma as previously described.[1] By determining the concentration of DS remaining per 100 ml. of maternal plasma at 60, 120, 180, 240, 300, and 360 minutes (Fig. 1), typical disappearance curves were established and from these disappearance curves the metabolic clearance rate of DS was calculated from the "slow" component of the curve, as described by Gurpide and associates.[2]

In addition to calculating the metabolic clearance rate from the disappearance slope, the apparent volume of distribution of DS (AVD_{DS}) was calculated by estimating the intercept at time zero.[2]

In these acute studies, immediately following the collection of the 180 minute sample of blood, pharmacologic manipulations were initiated. After the desired pharmacologic change was made, plasma samples were drawn at 20 to 40 minute intervals in order to establish a new disappearance curve (Figs. 2 and 3). In this manner, the acute effect of a pharmacologic agent or treatment regimen was evaluated as to its effect on the MCR_{DS} and the AVD_{DS} following a single injection of ^{3}H-DS.

The utility of this type of study was initially established by evaluating the effect of angiotensin II (A-II)–induced hypertension upon the MCR_{DS} in two normotensive term pregnant volunteers without renal or cardiovascular disease. After the 3 hour plasma sample was drawn, the patient's diastolic blood pressure was elevated 20 mm. Hg and held at this level by a continuous infusion of A-II for 2 hours. The maximum diastolic blood pressure achieved was 86 mm. Hg in Subject G. J. (Fig. 2). During the pressor response, multiple blood samples were drawn and a new disappearance curve established. In this manner, two separate straight lines of disappearance were obtained, that is, a disappearance curve was established before and after administration of A-II (Figs. 2 and 3).

With the same technique, the acute effects of hydralazine hydrochloride and furosemide also were measured. In the studies utilizing hydralazine, all patients were term or near-term severe chronic hypertensive subjects in whom delivery and control of blood pressure was mandatory; in all cases, these studies were conducted within 48 hours of delivery.

At the time of study, patients were placed in the left lateral position and injected with a single dose of ^{3}H-DS and several samples were collected during the first 180 minutes as described above. Following this, a dilute solution of hydralazine (0.2 mg. per milliliter) was administered intravenously by a Harvard Infusion Pump* until the diastolic blood pressure was in the desired therapeutic range of approximately 90 mm. Hg. Care was taken to monitor maternal pulse and keep it at or below 120 per minute. Fetal heart tones were also monitored throughout the infusion period and no fetal tachycardia was noted. Once the desired diastolic blood pressure had been established (90 mm. Hg), blood samples were collected at 20 to 30 minute intervals.

The same technique was applied next in five chronic hypertensive near-term pregnant volunteers. Three hours after the administration of ^{3}H-DS, a single intravenous injection of furosemide, 40 mg., was administered and blood samples were collected at 30 minute intervals to establish a second disappearance curve. Maternal blood pressure and pulse as well as fetal heart tones were monitored throughout the study and in no instance was a decrease in maternal blood pressure observed or an instance of fetal distress noted.

*Model 975, Harvard Appartus Co., Inc., Millis, Massachusetts.

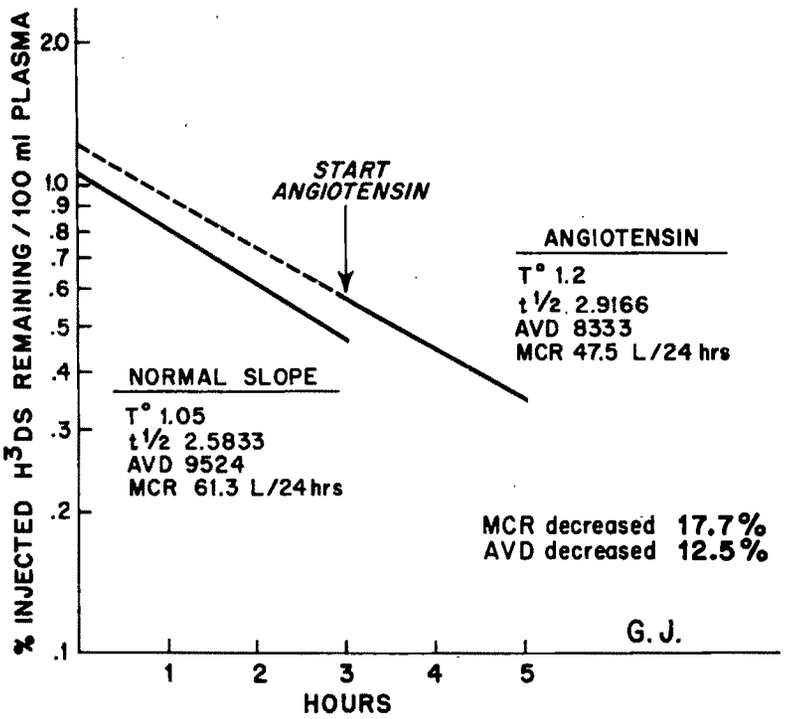


Fig. 2. The effect of angiotensin II on the metabolic clearance rate of dehydroisoandrosterone sulfate. The lower line represents the normal slope of disappearance following intravenous injection of ^{3}H-DS. T^0 indicates the projected intercept of the line at time zero and $t½$ indicates the half-time. *AVD* indicates the apparent volume of distribution of DS in milliliters and *MCR* the metabolic clearance rate of DS in liters per 24 hours. The upper line represents the disappearance slope established during an angiotensin II infusion.

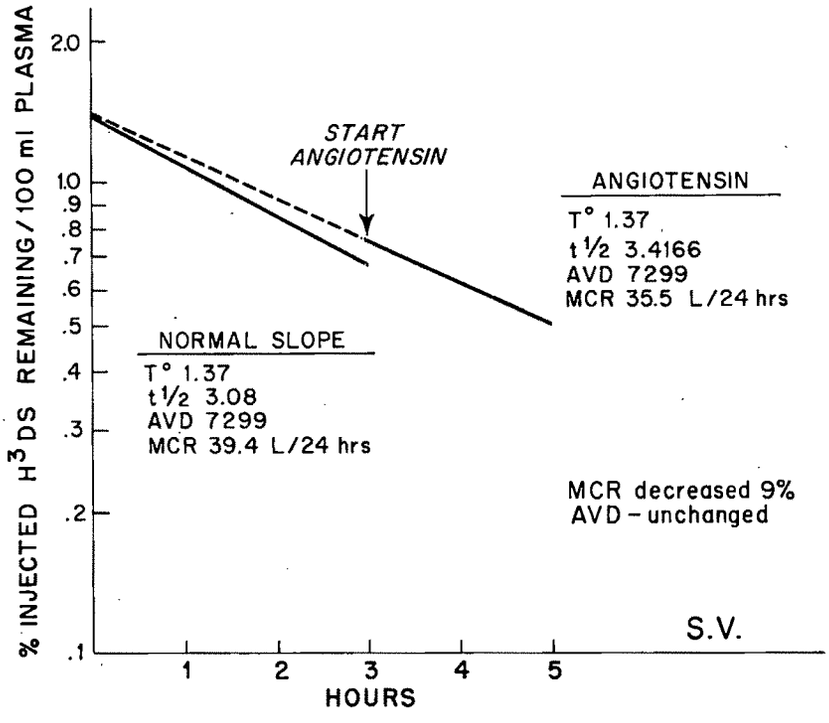


Fig. 3. The effect of angiotensin II on the metabolic clearance rate of dehydroisoandrosterone sulfate. The disappearance slopes and legends are the same as in Fig. 2.

Table I. Individual patient values for the metabolic clearance rate of dehydroisoandrosterone sulfate (MCR_{DS}) and the apparent volume of distribution of DS (AVD_{DS}) before and during therapy with hydralazine hydrochloride in gravidas with chronic hypertension

Patient	MCR_{DS} (L./24 hr.)		Change (%)	AVD_{DS} (ml.)		Change (%)	Change in diastolic blood pressure (mm. Hg)
	Before therapy	During therapy		Before therapy	During therapy		
A. S.	68	60	↓11.8	8,130	9,347	↑15.0	↓44
C. F.	66	59	↓10.6	9,615	12,980	↑35.0	↓44
J. C.	63	32	↓49.2	9,434	14,084	↑49.3	↓26
O. W.	46	19	↓58.7	6,060	9,700	↑60.1	↓30
F. D.	36	34	↓ 5.6	10,752	7,810	↓27.4	↓40
C. R.	50	35	↓30.0	8,333	6,896	↓17.2	↓40
J. A.	41	37	↓ 9.8	9,090	7,570	↓16.7	↓35
B. W.	69	60	↓13.0	13,510	12,650	↓ 6.4	↓40
Mean	54.9	42.0	↓23.5	9,365.5	10,129.6	↑ 8.1	↓37.4

Table II. Individual patient values for the metabolic clearance rate of dehydroisoandrosterone sulfate (MCR_{DS}) and the apparent volume of distribution of DS (AVD_{DS}) before and during therapy with furosemide in gravidas with chronic hypertension

Patient	MCR_{DS} (L./24 hr.)		Change (%)	AVD_{DS} (ml.)		Change (%)	Change in diastolic blood pressure (mm. Hg)
	Before therapy	During therapy		Before therapy	During therapy		
A. F.	86.0	74.0	↓13.9	12,500	11,111	↓11.1	0
C. B.	63.0	50.0	↓20.6	8,474	12,048	↑42.2	0
M. B.	64.0	51.0	↓20.3	6,097	6,666	↑ 9.3	0
O. I.	55.5	43.3	↓22.0	8,620	10,416	↑20.8	0
J. B.	58.5	47.0	↓19.6	7,042	8,474	↑20.3	0
Mean	65.4	53.1	↓18.8	8,546.6	9,743	↑14.0%	

Results

Angiotensin II studies. In both studies utilizing A-II–induced hypertension in normal term pregnant subjects, the MCR_{DS} was decreased (Figs. 2 and 3). The decreases observed were 9 and 17.7 per cent below control values, for a mean decrease of 13 per cent. The largest decrease occurred in Subject G. J., who also had the highest elevation of blood pressure throughout the infusion period.

The only change in AVD_{DS} occurred in Subject G. J. who, as previously mentioned, had the highest level of A-II–Induced blood pressure change. In this patient, the AVD_{DS} was decreased 12.5 per cent.

Hydralazine hydrochloride studies. The results obtained in eight near-term chronic hypertensive gravidas, following hydralazine therapy, are shown in Table I. The MCR_{DS} decreased in all patients studied, with a mean decrease of 23.5 per cent and a range of 5.6 to 58.7 per cent. Although the apparent volume of distribution was increased in four studies and decreased in the other four, the mean values indicate an increase of 8.1 per cent over control values. The mean decrease in diastolic blood pressure in these subjects was 37 mm. Hg, with a range of 26 to 44 mm. Hg. In each instance the diastolic blood pressure was not lowered below 90 mm. Hg.

Furosemide studies. The acute effects of 40 mg. of furosemide administered intravenously to five near-term chronic hypertensive gravidas are shown in Table II. The MCR_{DS} decreased in all patients studied despite a lack of blood pressure change during the course of the study. The average decrease in MCR_{DS} was 18.8 per cent, with a range of 13.9 to 22 per cent. The AVD_{DS} surprisingly increased in four of the five subjects studied, with a mean increase of 14 per cent.

Comment

An increase in diastolic blood pressure of 20 mm. Hg in the two normotensive patients studied resulted in a decrease in the MCR_{DS} in both subjects. Interestingly, the greatest decrease in MCR_{DS} occurred in the patient who had the highest elevation in diastolic blood

pressure (Subject G. J.). In this patient, the blood pressure never was allowed above 86 mm. Hg, but the elevation from 66 to 86 mm. Hg resulted in a greater than 17 per cent decrease in MCR_{DS}. Although it is not possible to make absolute comparisons between this form of artifically induced hypertension and the hypertension which occurs in chronic essential hypertension or pre-eclampsia, nevertheless, the increase in diastolic blood pressure induced by antiotensin II suggests that similar increases in diastolic blood pressure occurring as a result of disease processes might also decrease functional placental reserve.

In Subject G. J., the AVD_{DS} decreased 12.5 per cent and, although the AVD_{DS} does not correspond to blood volume, recent studies in this laboratory suggest that the AVD_{DS} corresponds with total albumin space.[3] Thus an acute decrease in AVD_{DS} may represent a sudden decrease in vascular volume induced by intense and prolonged (2 hour infusion) arteriolar spasm.

The utilization of hydralazine hydrochloride as an antihypertensive agent to treat hypertensive complications of pregnancy has been widespread in the United States. The choice of this agent during pregnancy has been based largely upon the assertion that it does not decrease renal or cerebral blood flow and increases cardiac output and oxygen consumption.[4, 5] The effect of hydralazine hydrochloride on utero-placental perfusion remains unsettled. Johnson and Clayton,[6] using clearance of ^{24}Na from myometrium, reported an increase in clearance after hydralazine therapy, but Dixon and colleagues,[7] using the same technique, observed no improvement in clearance of ^{24}Na from myometrium. Using a more direct measurement of uterine blood flow in the sheep, Lader and associates[8] observed a decrease in uterine blood flow proportional to the decrease in maternal blood pressure. A similar decrease in ovine uterine blood flow was reported also by Greiss and Wilkes.[9]

In the present study, the MCR_{DS} was decreased in all eight subjects studied, with a range of 5.6 to 58.7 per cent following the use of hydralazine hydrochloride. That these decreases in MCR_{DS} represented, at least, in part, a decrease in placental function is illustrated by the study in Patient O.W. (Table I).

O. W. was a 25-year-old black woman, gravida 3, para 0, who was admitted near term with a blood pressure of 180/120. The outlined therapy resulted in a decrease in this patient's diastolic blood pressure to 90 mm. Hg. Interestingly, the MCR_{DS} decreased from 46 to 19 L. per 24 hours, a decrease of 58.7 per cent, following the 30 mm. Hg decrease in diastolic blood pressure. Two days later, at cesarean section, a viable infant was delivered. The placenta had a large infarcted area covering approximately 40 per cent of the surface area. In this patient, with at least 40 per cent of functional placental area already compromised, lowering the diastolic blood pressure to 90 mm. Hg resulted in a severe compromise of placental function. This observation strongly supports the contention that the measurement of MCR_{DS} is, at least in part, reflective of perfusion in the placenta. Of equal importance, this observation displays that even in severe hypertension the placenta manifests a marked degree of functional reserve. In instances where this functional reserve has been compromised already, as was evident in this patient, a further reduction in placental perfusion may markedly jeopardize the fetus.

The rapid-acting and extremely potent diuretic furosemide induced a decrease in the MCR_{DS} in all subjects studied despite a failure of blood pressure to decrease in any of the subjects studied. In addition, despite its well-known diuretic effect, this agent surprisingly did not decrease the apparent volume of distribution. In fact, the AVD_{DS} increased in four of the five subjects studied, resulting in a mean increase in AVD_{DS} of 14 per cent, a result contrary to what intuitively might have been expected following volume depletion. The results of this study suggest that furosemide may have a direct effect on vascular smooth muscle as well as its well-recognized diuretic effect. Certainly the decrease in MCR_{DS}, with no change in blood pressure and the accompanying increase in the AVD_{DS}, suggest that the decrease in MCR_{DS} observed in all five of these subjects was the result of alterations in perfusion of the placenta and/or liver.

The present study indicates that caution should be exercised before and during administration of antihypertensive or potent diuretic agents to hypertensive pregnant women. Specifically, it is probably unwise to reduce maternal diastolic blood pressure to below 100 mm. Hg if fetal viability is a major consideration. In addition, it seems unwise to utilize a potent diuretic agent like furosemide except in a life-threatening situation such as pulmonary edema. Unless these rather specific indications are considered, the use of these pharmacologic agents may adversely effect functional placental reserve and jeopardize our second patient, the fetus.

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Maternal mortality rates in eclampsia

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This study evaluates the dominant factors that influence the course of eclampsia toward a lethal outcome by means of statistical analysis of various clinical, laboratory, and morphologic data of 365 cases of toxemia with convulsions, that included 49 deaths and 33 autopsies, registered during a 9 year period. This communication suggests that the death of eclamptic patients resulted from a combination of several factors that showed a very wide individual variation. Since advancing age of the mother was associated with a high incidence of coincidental renal and vascular diseases, this was the most clearly influential factor. The presence of twins, delay in hospitalization, undue haste in deciding upon cesarean delivery, and underestimation of the initial clinical condition were all influential factors, although without statistical significance. Cesarean section, performed in 141 instances of ante- and intrapartum eclampsia (40.4 per cent), was not associated with a higher maternal mortality rate.

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THE MATERNAL mortality rate in eclampsia varies from 0 to 20.0 per cent in contemporary medical literature,[1, 12] even though most authors pursue similar general therapeutic objectives. Furthermore, many modern managements have not been able to improve significatively, and sometimes not even duplicate, the results reported by Stroganoff[15] in the early part of this century. This fact becomes a flagrant contradiction in view of the spectacular medical progress seen in the last 50 years.

Eden,[3] Stroganoff,[15] and Peckham[11] recognized important differences in the clinical picture of eclamptic women. Some cases would follow a rather benign uncomplicated course, with prompt recovery, while others would frequently end in death. It became evident that any rational therapy could give optimal results in the first type of patients and be relatively unsuccessful with the second group. Thus, without underestimating the importance of a correct therapeutic approach that requires expertly balanced individualization,[17] we consider that it was imperative to try to identify the dominant factors that may influence the course of the disease toward a lethal outcome independent of therapeutic considerations.

The death of a patient may be viewed as the result of a variable association of factors within a limited clinicopathologic and therapeutic spectrum. The simultaneous or successive analysis of multiple factors has met with serious conceptual and programmatic difficulties. Its utility depends on the proper selection of variables, on the possibility of quantitative estimation of corresponding parameters, and on the existence of significant differences in the data under study.

The present report, based on analysis of various clinical, laboratory, and morphologic data, attempts to identify and evaluate the major factors that influence the course of eclampsia toward a low or high maternal mortality rate.

Clinical material

All maternal deaths due to toxemia of pregnancy and its direct complications, registered at the Hospital de Gineco-Obstetricia No. 2 del Centro Médico Nacional during the 9 years ending on Dec. 31, 1973, formed the material of the present study. Toxemic women who died of coincidental diseases or obstetric complications unrelated to the toxemic process and its management were not included in this analysis. Hence, the natural history of the disease, influenced only by the conventional therapeutic measures recommended

From the Hospital de Gineco-Obstetricia No. 2 del Centro Médico Nacional del I. M. S. S.

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Table I. Maternal mortality rate in antepartum, intrapartum, and postpartum eclampsia

	Antepartum eclampsia (n = 189)	*Intrapartum eclampsia (n = 110)*	*Postpartum eclampsia (n = 66)*	*Total cases (n =365)*
Maternal mortality rate (per cent)	16.9	10.0	9.1	13.4

Chi square = 4.14 (N.S.).

Table II. Maternal mortality rate in early eclampsia

	21-29 wks. (n = 28)	*30-44 wks. (n = 335)*	*Total cases (n = 363)*
Maternal mortality rate (per cent)	28.5	12.2	13.5

Chi square = 5.89 ($P < 0.02$).

by the "toxemia clinic" throughout this period,[7, 9] was evaluated.

Maternal age, parity, amenorrhea, blood pressure, proteinuria, number of convulsions, type of delivery, blood chemistries, plasma proteins, complete clinical diagnosis, and main postmortem findings were the parameters studied. Chronologic aspects such as timing of admission, convulsions, delivery, and laboratory sampling were also considered. A similar analysis was carried out in a comparable group of patients surviving the eclamptic episode. Most of these parameters were subjected to simple descriptive statistical techniques and, when feasible group data received comparative processing.

Analysis of the data

Antepartum, intrapartum, and postpartum eclampsia. Table I presents maternal deaths in eclamptic women divided according to the classical concept of antepartum, intrapartum, and postpartum cases. Although antepartum eclampsia showed the highest mortality rate (16.9 per cent), the differences were not significant. This fact, however, does not rule out entirely the greater risk of antepartum eclampsia, since the differences would become significant if the same tendencies were observed in a population twice the size of the present study.

Early eclampsia. The greater risk of early antepartum eclampsia becomes distinct if the patients are divided in two groups depending on whether convulsions took place before or after the thirtieth week of amenorrhea (Table II). If the cases are further divided by 5 week intervals, the significance is smoothed out as a consequence of the smallness of the first two groups (Table III). Thus, excepting the group of very early eclampsia, the timing of convulsions in regard to the length of gestation does not seem to be of great importance as far as maternal mortality rates are concerned, since the figures become similar after the thirtieth week of amenorrhea.

Clinical and laboratory data in survivors and nonsurvivors of eclampsia. Table IV presents the main characteristics of ante- and intrapartum eclampsia depending on maternal outcome. Postpartum eclampsia cases were not included since they differ in various clinical aspects and pose relatively different therapeutic problems. Blood samples for laboratory work were taken within the first 12 hours after admission in 45.5 per cent of the cases and within the second 12 hour period in the remaining cases. Since there were no statistically significant differences in timing this factor was disregarded under the circumstances of the present study.

As a whole, the eclamptic patients who died showed more severe clinical and laboratory data than those that survived, but significant differences existed only in the magnitude of hypertension and in the age of the patients. The differences in the total number of convulsions and in the levels of blood creatinine were only probably significant. Proteinuria and the levels of blood urea were higher in the group of nonsurvivors and the differences would have reached the significance level if the series were at least two thirds larger. The degree of hypoproteinemia, and particularly of hypoalbuminemia, that characterized these patients is noteworthy, even though there were no differences between survivors and nonsurvivors. The importance of metabolic and respiratory acidosis in eclampsia is well indicated by the levels of blood CO_2 found in the present study, even though this condition is much better evaluated with complete acid-base balance studies. Thus, in spite of several limitations, the synthesis of these data seems to indicate a more severe vasospasm with worse repercussions in the renal, cerebral, and metabolic systems in the nonsurvivors, who were significantly older than the survivors.

Eclampsia in teenagers and in older women. If the main clinical and laboratory characteristics of a group of 73 cases of teenage ante- and intrapartum eclampsia

Table III. Gestational amenorrhea and maternal mortality rate in eclampsia

	21-24 wks. (n = 6)	*25-29 wks. (n = 22)*	*30-34 wks. (n = 61)*	*35-39 wks. (n = 196)*	*40-44 wks. (n = 78)*	*Total cases (n = 363)*
Maternal mortality rate (per cent)	16.7	31.8	9.8	13.3	11.5	13.5

Chi square = 7.3 (N.S.).

Table IV. Comparison of main clinical and laboratory data between survivors and nonsurvivors of ante- and intrapartum eclampsia

	Ante- and intrapartum eclampsia		
	Deaths	*Survivors*	*P**
Maternal age (yr.)	27.5 ± 0.95 n = 43	24.9 ± 0.43 n = 255	<0.025
Previous parity	2.5 ± 0.46 n = 41	1.7 ± 0.17 n = 256	N.S.
Amenorrhea (wk.)	35.5 ± 0.71 n = 40	36.2 ± 0.29 n = 243	N.S.
Highest systolic B.P. (mm. Hg)	190.5 ± 3.94 n = 43	181.7 ± 1.69 n = 256	<0.05
Highest diastolic B.P. (mm. Hg)	130.9 ± 3.26 n = 43	122.1 ± 1.02 n = 256	<0.025
Average systolic B.P.† (mm. Hg)	170.5 ± 3.31 n = 43	158.7 ± 1.06 n = 256	<0.001
Average diastolic B.P.† (mm. Hg)	116.6 ± 2.45 n = 43	107.2 ± 0.65 n = 256	<0.001
Highest proteinuria (Gm./L.)	6.4 ± 1.33 n = 39	4.65 ± 0.36 n = 248	N.S.
No. of convulsions	7.13 ± 1.07 n = 38	4.88 ± 0.25 n = 253	<0.05
Total serum proteins (Gm./100 ml.)	4.9 ± 0.19 n = 16	4.8 ± 0.04 n = 196	N.S.
Serum Albumin (Gm./100 ml.)	2.5 ± 0.22 n = 16	2.5 ± 0.03 n = 196	N.S.
Blood urea (mg./100 ml.)	40.3 ± 5.06 n = 35	30.8 ± 1.2 n = 217	N.S.
Blood creatinine (mg./100 ml.)	2.0 ± 0.36 n = 22	1.25 ± 0.045 n = 189	<0.05
Blood CO_2 (mEq./L.)	19.3 ± 0.84 n = 31	20.2 ± 0.17 n = 209	N.S.

*Student's t test.

†Six hours time.

with a total maternal mortality rate of 5.5 per cent are compared with those of 89 women after 30 years of age with a maternal mortality rate of 20.0 per cent ($P < 0.005$), only two statistically significant differences are observed. The highest and the average systolic blood pressure readings are lower in the teenage eclamptic patients (175.5 and 153.9 mm. Hg, respectively, vs. 188.3 and 164.7 mm. Hg in the older patients: $P < 0.005$). Even these significant differences may lose their importance since older women show a physiologic tendency for higher blood pressure readings than their younger peers. The younger eclamptic patients may have experienced worse vasoconstriction, despite their lower blood pressure readings, since they started from a lower normal age-related blood pressure level. These clinical and laboratory similarities between two relatively extreme groups point out the importance of age in the maternal outcome of eclampsia. Furthermore, two of the four deaths in teenagers occurred in twin pregnancies, whereas there

Table V. Age groups and maternal mortality rate in eclampsia

	15-24 yr. (n = 203)	*25-34 yrs. (n = 118)*	*35-46 yrs. (n = 43)*	*Total cases (n = 364)*
Maternal mortalality rate (per cent)	8.9	19.5	18.6	13.5

Chi square = 8.33 ($P < 0.02$).

were none among patients after 30 years of age (see paragraph on twin pregnancies and following discussion). If only single pregnancies are considered in these two age groups, the maternal mortality rates become 2.9 and 21.2 per cent, respectively ($P < 0.001$).

Age and maternal death in eclampsia. Table V presents maternal mortality figures in eclamptic women divided in three groups according to age. The differences are statistically significant and it is evident that they result from a lower mortality rate in the youngest group. A further correlation of death and maternal age in 11 groups with spans of 3 years gives a Pearson's coefficient of 0.766 ($P < 0.01$ with 9 degrees of freedom) which indicates a significant positive correlation between these parameters.

Maternal death and clinical data in cesarean and noncesarean cases of ante- and intrapartum eclampsia. Previous parity was the only parameter showing significant differences in these groups (1.4 and 2.4, respectively: $P < 0.005$). The figures for maternal deaths (14.9 and 13.9 per cent, respectively), maternal age, amenorrhea, highest and average blood pressure, proteinuria, and number of convulsions were almost all identical. The laboratory data for total serum proteins, serum albumin, blood urea, creatinine, and CO_2 did not show any significant differences.

The 21 deaths in the group of cesarean operations included at least 9 cases in which the operation was performed after the diagnosis of extensive brain hemorrhage was established in the mother, in order to offer the fetus a chance for survival. Actually, only two fetuses were lost in these nine cases that included two sets of twins. On the other hand, the 22 deaths in the group without cesarean operation included nine cases that died undelivered, with only two fetal survivals in three instances of postmortem cesarean sections. If these types of deaths are exluded from their respective groups, neither the mortality differences nor the rest of clinical and laboratory data change substantially.

Eclampsia in twin pregnancy. There were 18 twin pregnancies in the total group of 365 cases of eclampsia. The incidence of multiple pregnancy in eclampsia was about three times higher than in the general population, and its maternal mortality rate was double the figure of the whole eclampsia group (27.8 vs. 13.5 per cent). Extensive brain hemorrhage accounted for three of the five maternal deaths in this high-risk group in spite of their relatively young ages (19, 19, 21, 23, and 24 years). This higher mortality rate was associated with lesser degrees of clinical and laboratory abnormalities than those presented in Table IV, with the exception of total serum proteins and serum albumin that reached the lowest levels in the present study (4.53 ± 0.58 and 2.26 ± 0.53 Gm. per 100 ml., respectively). As a consequence of the small number of twin pregnancies registered, the differences were not significant, and it is doubtful that their higher mortality rate could affect the over-all mortality figures, since their incidence was only 4.9 per cent of all eclamptic cases. As a matter of fact, if the 18 twin pregnancies are excluded, the maternal mortality rate drops only 0.7 per cent.

Intercurrent eclampsia and delayed delivery cases. There were six patients with intercurrent eclampsia who gave birth to surviving premature fetuses and nine patients with early antepartum eclampsia who improved rapidly after the fetus died "in utero," but delivery did not take place for several days. The average interval between the first convulsion and the time of delivery in these 15 cases was 15.7 ± 10.3 days. There were no maternal deaths in these atypical cases, and their clinical and laboratory characteristics when compared with the group of survivors in Table IV showed only two significant differences; these patients were older (29.7 ± 1.76 years, $P < 0.01$) and had fewer convulsions (3.07 ± 0.68, $P < 0.025$) than the rest. Another interesting fact of this type of eclampsia was its early presentation since the average amenorrhea was 32.2 ± 2.26 weeks, but due to the small size of the series this difference was not significant. This clinical behavior seemed to indicate a very prompt response to treatment made possible by the rapid death of the fetus in some cases and/or by an unusual reversibility of the pathologic process.

Chronology of main events. Convulsions began before admission to the hospital in 171 patients with ante- and intrapartum eclampsia (57.2 per cent of the group) and on admission or shortly thereafter in 128

Table VI. Main postmortem findings in 33 eclamptic patients before and after 30 years of age

	Before 30 yr. (n = 19)	*30 yrs. and up (n = 14)*	*Totals (n = 33)*
Kidney:			
Toxemic lesion	19 (100.0)*	13 (92.9)	32 (97.0)
Acute tubular necrosis	3 (15.8)	0	3 (9.1)
Chronic glomerulonephritis	0	1	1
Nephrosclerosis	0 (31.6)	1 (57.1)	1 (42.4)
Chronic pyelonephritis	6	6	12
Double and triple renal arteries	1 (5.3)	2 (14.3)	3 (9.1)
Heart:			
Average weight (Gm.)	310.9	336.1	322.3
Left ventricle thickness (mm.)	12.4	13.0	12.7
Cardiac hypertrophy	12 (63.1)	14 (100.0)	26 (78.8)
Brain:			
Parenchymatous hemorrhages	10 (52.6)	9 (64.3)	19 (57.6)
Infarction and softening	0	1 (7.1)	1 (3.0)
Acute hypoxic encephalopathy	2 (10.5)	1 (7.1)	3 (9.1)
Edema (only)	4 (21.0)	2 (14.3)	6 (18.2)
Normal	3 (15.8)	0	3 (9.1)
Condition unknown	0	1 (7.1)	1 (3.0)
Liver:			
Average weight (Gm.)	1,847	1,980	1,907
Subcapsular hematoma	1 (5.3)	2 (14.3)	3 (9.1)

*Numbers in parentheses are percentages.

cases. The maternal mortality rates were very similar in both groups—14.03 and 14.8 per cent, respectively.

Eighty cases of convulsions before admission and cesarean delivery had a maternal mortality rate of 12.5 per cent; and in 76 similar noncesarean cases this figure was 18.4 per cent. On the other hand, 59 patients with eclampsia after admission and cesarean delivery had a mortality rate of 18.6 per cent; and in 69 similar vaginal delivery cases this figure was 11.6 per cent. However, if the 12 patients that were considered moribund on admission, nine cases of cesarean operation with preoperative diagnosis of cerebral hemorrhage, and four instances of cesarean delivery due to obligatory indications (three cases of severe abruptio placentae and one case of previous section) are excluded, the corrected maternal mortality rates become 4.1, 8.8, 9.4, and 6.1 per cent, respectively, in the previous four subgroups.

When the time intervals between the beginning of convulsions and admission to the hospital and eventual vaginal or cesarean delivery are considered, the group with eclampsia before admission has two important observations. The period between the first convulsion and the moment of arrival at the hospital is longest in the nonsurvivors with vaginal delivery (6.85 ± 6.36 hours), and the interval between admission and delivery in the group of survivors with cesarean operation is the shortest (10.5 ± 9.8 hours).

When a similar analysis is made in the group with convulsions starting after admission, the period between the first convulsion and the time of delivery in the nonsurvivors with cesarean section is the shortest (6.23 ± 5.25 hours), but this group also shows the longest interval between arrival and delivery (20.5 ± 26.1 hours). None of the three differences is statistically significant.

Morphologic data. Table VI presents the main postmortem findings in a total of 33 eclamptic women, divided into two groups according to age. Parenchymatous brain hemorrhages were found in 57.6 per cent of the cases. Microscopic evidence of chronic pyelonephritis, chronic glomerulonephritis, and/or nephrosclerosis existed in 42.4 per cent of the patients. The toxemic renal lesion was evident in all cases but one, that of a patient with chronic glomerulonephritis in whom the signs of a superimposed toxemia would have required electron microscopy studies for definite demonstration. There were three instances of subcapsular liver hemorrhages, three of acute tubular necrosis, and 10 patients with brain changes other than hemorrhage but important enough to jeopardize their survival. Finally, the weights of the liver and of the heart were abnormally increased in most cases.

Since the average height of 107 survivors of eclampsia from the same obstetric population was 150.7 ± 6.1 cm., with a corresponding calculated average weight of the heart of 247 ± 30 grams,[16] cardiac hypertrophy in the autopsy cases was defined as a weight greater than 307 grams and/or a left ventricle thickness of 12 mm. or more. Morphologic

evidence of cardiac hypertrophy was present in 78.8 per cent of the cases.

As a whole, patients 30 years of age or more (n = 14) showed more pathologic changes and more coincidental diseases than younger women (n = 19). This was noticeable in most organs, but it was more evident in the frequency of coincidental renal pathology and in the frequency and magnitude of cardiac hypertrophy. None of the differences was statistically significant.

Comment and conclusions

The data presented in this study seem to indicate that the death of eclamptic patients resulted from the combination of several factors that show wide individual variability. Only a few patients with clear-cut clinical, laboratory, and morphologic evidence sometimes present a single dominant factor as the cause of death, and this is often found to be gross mishandling of the case by inexperienced medical and paramedical personnel.

Contrary to logical expectations, statistical analysis in most instances was not capable of defining satisfactorily the importance of those potential influences. This was an inevitable consequence of the size of the series and of its necessary fragmentation for the evaluation of chosen variables under different circumstances. In spite of the relatively small number of cases, in certain groups, some factors showed a significant association with the final outcome of eclamptic women and others seemed to be indifferent. *The most clearly influential factor was the age of the patient at the time of eclampsia.* Since this complication occurs preferentially in young primigravid women whose ages show a narrow range, the prognostic importance of aging has seldom been detected.[1, 2, 5, 7, 10] Age per se, however, within the increasingly shorter reproductive span of modern woman, does not show a very clear effect in most gestational pathology. What it most likely indicates is an over-all loss of optimal efficiency in the essential homeostatic mechanisms that reduces the adaptability of vital systems to various types of pathogenic influences and stress.

On the other hand, relative aging and pregnancy may be associated with the emergence of previously nondetectable underlying pathology. An increase in age, even within a narrow range, may be accompanied by general and regional changes that potentiate each other reciprocally, and in the proper pathogenic setup render the individual more prone to fatal consequences. The fact that very early eclampsia, that is frequently associated with underlying disease, showed a significantly higher maternal mortality rate, is in keeping with this interpretation.

The postmortem studies of 33 eclamptic patients detected two important facts: a high incidence of chronic pyelonephritis and cardic hypertrophy. Whether the first finding was merely coincidental and had no influence on the pathogenesis of eclampsia cannot be ascertained at the present time, but it must be carefully assessed in view of their frequent association.[6] Furthermore, according to Freedman,[4] the incidence of nonobstructive chronic pyelonephritis found at autopsy varies from 0.6 to 2.4 per cent, and Sheehan and Lynch[14] reported an incidence of "large pyelonephritic" scars in 5.7 per cent of autopsied toxemic and eclamptic women. These figures are several times smaller than those found in the present report. Even though it is a fact that urinary infections thrive among poor people, to which group toxemic patients usually belong, one must keep in mind the limitations for establishing the role of bacterial infections on the basis of morphologic information alone[4] and the possible differences in diagnostic criteria among authors.

As far as cardiac hypertrophy is concerned, the paramount question is whether it resulted from the toxemic episode alone or whether it existed prior to pregnancy. The fact that it was more frequent and more marked in women after 30 years of age would seem to support the latter possibility, but the lack of statistical significance in the differences among age groups and our limited knowledge about the chronologic and hemodynamic requirements for early left cardiac hypertrophy make uncertain any interpretation.

In a group of 67 eclamptic deaths among English and Scottish women, Sheehan and Lynch[13] found an incidence of 25.4 per cent of hearts weighing more than 340 grams, and estimated that at least two thirds of them were due to chronic hypertension. If this same criteria is followed in the present study, the total incidence of abnormally heavy hearts becomes 29.0 per cent, with a striking difference between young and older women (17.6 vs. 42.8 per cent), although no statistical significance existed because of the size of the series. The figures used by Sheehan and Lynch are not suitable for the present communication in view of the important differences in stature between English and Scottish and Mexican women. Thus, the top limit of 307 grams, that resulted from Zeek's[16] average plus two standard deviations for an average height of 151 cm., seems more applicable to an eclamptic Mexican population and, as such, a remarkable majority of these patients presented indisputable evidence of important left cardiac hypertrophy, possibly associated with preclinical and undetected chronic hypertension or

chronic anemia and malnutrition, in an at present undefinable percentage of the cases.

The previous clinical and morphologic description and discussion provide strong evidence for stating that the eclamptic population of the present study included a remarkably high proportion of coincidental renal and vascular diseases that jeopardized their most vital homeostatic mechanisms. This association increased with advancing age of the patients and led to a significantly higher maternal mortality rate in older women. A similar conclusion had been reached after analysis of a smaller group of eclamptic women.[8]

Eclampsia in twin pregnancies had a higher mortality rate despite the young ages of these patients, and with lesser degrees of clinical and laboratory abnormalities than those found in the total group of survivors. This may indicate that the hemodynamic and obstetric characteristics of twin pregnancy per se accentuate the consequences of the toxemic condition by decreasing even more the homeostatic capabilities of the whole organism. The fact that this group showed the lowest values of serum proteins of all eclamptic cases and that three out of five deaths in young women were due to cerebral hemorrhage may support this latter view.

Cesarean section in this study of eclamptic women was not associated with a higher maternal mortality rate if nine cases with the diagnosis of extensive brain hemorrhage prior to surgery and four instances of surgical delivery because of obligatory indications are excluded. When the chronology of events is considered and the individual records are revised, one gets the impression that the cesarean operation was decided upon too soon after a convulsion and before adequate control of the condition in two patients, but its influence on the fatal outcome of these cases remains difficult to assess.

The mortality figures found in the different groups correlated with chronologic data agree with the logical opinion stating that prompt hospitalization and well-timed management are important prognostic factors in eclamptic patients. In this context, the fact that 42.8 per cent of ante- and intrapartum eclampsia patients had their first convulsion on admission or shortly thereafter, is a most disturbing finding. Thirty-three patients began convulsions while in the admitting department and/or within the first hour of hospitalization, before therapy could be effective. The remaining 95 (31.7 per cent of the total) began convulsing at an average of 9.2 hours after being admitted for three possible reasons: (1) these patients had a rapidly progressive type of toxemia; (2) the stress of labor, if present, increased the severity of the disease; and/or (3) their clinical condition was underestimated, a common error when inexperienced personnel handle toxemic women.[5] Clarification of the relative significance of each one of these three possibilities goes beyond the scope of the present communication, but it is obvious that, if convulsions and their often fatal consequences are to be largely prevented, no single case of pre-eclampsia should be underestimated.

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Effects of epinephrine on distribution of blood flow in the pregnant ewe

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Seven pregnant ewes ranging from 85 to 140 days of gestation were infused with systemic doses of epinephrine and uterine arterial flow dose-response curves were determined. With a constant systemic infusion of epinephrine at a mean rate of 0.29 ± 0.03 μg/Kg. · min., and the radionuclide labeled microsphere method to measure arterial blood flow, a 38.5 per cent decrease in total uterine arterial blood flow was demonstrated while systemic pressure was unaltered. At this dose the reduction in endometrial blood flow was significantly greater (−58.7 per cent) than that in either the myometrium (−36.9 per cent) or placental cotyledons (−34.5 per cent) ($p < 0.025$ and < 0.005, respectively). There also occured a decrease in blood flow to the mammary gland and the pancreas, whereas increases in blood flow to the skeletal muscle, adipose tissue, and spleen were documented. It is evident from this study that during the period of ovine pregnancy investigated, the vascular beds of all tissues comprising the pregnant uterus, including the placental cotyledons, are sensitive to the vasoconstrictive effects of epinephrine.

THE RESPONSE of the uterine vascular bed to vasoactive agents is the subject of numerous studies. Previous studies[1–6] have demonstrated that adrenergic drugs can influence the magnitude of uterine blood flow. The presence of both alpha- and beta-adrenergic drug receptor sites has been demonstrated in the uterine vascular bed.[4]

Studies by Barton and associates[6] have also shown that similar concentrations of exogenous epinephrine and norepinephrine can cause similar reductions in uterine arterial flow when infused into the uterine artery. A 50 per cent reduction in uterine arterial flow occured with an intra-arterial concentration of exogenous epinephrine ranging from 2 to 10 ng. per milliliter of arterial blood. This study raised two further questions. (1) Were some components of the uterine circulation spared from the action of catecholamines? (2) If epinephrine caused significant vasoconstriction in the pregnant uterus at these low concentrations, what were the systemic effects of epinephrine in this same concentration range?

In order to answer these questions, we employed a chronic unanesthetized sheep preparation with implanted uterine artery flow probes. Radioactively labeled microspheres were infused systemically before and during an intravenous epinephrine infusion in order to determine the effect of epinephrine on the placental, myometrial, and endometrial circulation, as well as other vascular beds.

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Methods

The study was performed in seven pregnant ewes ranging from 85 to 140 days of gestation and included three singleton and four twin pregnancies. The preparation was the same as that described by Rosenfeld and associates[7, 8] consisting of pregnant animals with electromagnetic flow probes (Micron Instruments, Los Angeles, California) implanted around both uterine arteries and polyvinyl catheters (0.9 mm. inner diameter by 1.2 mm. outer diameter) in both femoral arteries passed to the trifurcation of the abdominal aorta and in a femoral vein passed to the high inferior vena cava. A radiopaque catheter (1.2 mm. inner

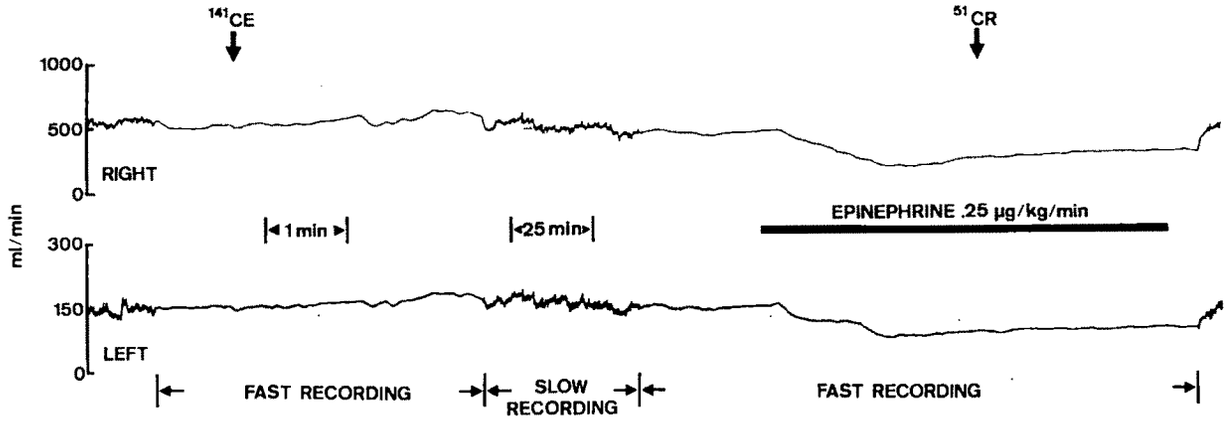


Fig. 1. Continuous flowmeter recordings of right and left uterine arteries in an ewe of 130 days of gestation carrying a singleton pregnancy on the right. The time of infusion of isotope-labeled microspheres is noted. The systemic infusion of epinephrine at 0.25 µg/min.·Kg. produced a lower steady flow in both uterine arteries.

diameter by 2.2 mm. outer diameter) was also passed via the left common carotid artery into the left ventricle.[7] The flow probes and inguinal catheters were carried out to the flank through a subcutaneous tunnel and were maintained in a canvas pouch fastened to the ewe's skin with steel pins. The left ventricular catheter was kept in a separate pouch attached to the neck. All catheters were flushed daily with heparinized saline (1,000 U. per milliliter).

Following surgery the ewes were maintained in stalls in the laboratory and studied after they had recovered from surgical trauma, generally within 4 days. Systemic arterial and venous pressures were monitored by pressure transducers connnected to a Gould amplifier (model N-4307-04, Cleveland, Ohio) and a two-channel Brush recorder (Model 220, Gould Inc., Cleveland, Ohio). Uterine blood flow was monitored with Micron Instruments electromagnetic flowmeters (Model RC 1000, Los Angeles, California) and recorded on another two-channel Brush recorder. The flow probes employed have a linear response to flows in the range studied and are provided with flow signal and zero flow calibrations.

The response of uterine arterial blood flow to systemic doses of epinephrine was investigated as follows.

Epinephrine solutions were freshly made by adding 4 ml. of 1:1000 epinephrine chloride (Adrenalin, Parke Davis) to 996 ml. of 5 per cent dextrose in isotonic saline, giving a concentration of 4 µg per milliliter of base, and used for no more than 4 hours at a time. This solution was infused with a Harvard infusion pump at varying rates through the inferior vena cava catheter. The period of infusion was 5 minutes for each rate examined, followed by a rest period of 10 to 15 minutes, permitting flows to return to preinfusion levels. No more than six infusions were performed per day. Simultaneous blood pressure, pulse rate, and uterine arterial blood flow measurements were obtained and electronically integrated. The speeds of the infusions were randomly chosen and dose-response curves constructed. A rate of epinephrine infusion was identified for each animal that caused a 30 to 40 per cent reduction of uterine blood flow. One to 2 days after determining the dose-response curve to systemic epinephrine infusion, the procedure was repeated, using the predetermined dose of epinephrine with the objective of infusing isotope-labeled microspheres into the left ventricle of the ewe before and during the constant epinephrine infusion (Fig. 1).

The method for the infusion and analysis of microspheres has been described in detail elsewhere[7] and is summarized here. Radioactive carbonized microspheres (Minnesota Mining and Manufacturing Co.) with ^{141}Ce and ^{51}Cr labels, a specific activity of 10mCi. per gram, and a mean diameter of 25 $\mu \pm 5$ S.D. were employed. The exact amount of microspheres in the infused suspension was determined by adding the microspheres, 8 to 10 ml. of sterile isotonic saline, and 1 drop of Tween 20 to a preweighed counting vial containing a magnetic stirring rod and reweighing the vials. The suspension was mixed on a magnetic stirer for 45 minutes and four 0.1 ml. samples of the suspension were removed in preweighed lengths of polyvinyl tubing while the mixing continued. These catheters were placed in preweighed counting vials, weighed, and counted. The mean counts per minute (c.p.m.) per gram of suspension were calculated. The suspension, whose weight and radioactivity per gram

Table I. Mean total counts per minute and number of microspheres infused into the study animals

Isotope	*Counts per minute*	*No.*
^{141}Ce:		
Mean	16.71×10^6	0.904×10^6
± S.E.M.	7.37×10^6	0.178×10^6
N.	7	7
^{51}Cr:		
Mean	12.52×10^6	1.826×10^6
± S.E.M.	3.24×10^6	0.368×10^6
N.	7	7

was known, was infused by air desplacement through the left ventricular catheter while the microspheres were kept in suspension with a stirring warming unit. The suspension was infused over 30 to 40 seconds and the ventricular catheter flushed with 10 ml. of isotonic saline. Afterward the entire administration unit was reweighed and the total counts per minute and number of microspheres infused were determined (see Table I). In the experiments reported here, the microspheres were infused before the epinephrine infusion and again during the epinephrine infusion. The latter microsphere infusion was not begun until the uterine blood flow, monitored by the implanted flowprobes, had reached a lower, steady rate of flow.

Starting just prior to the microsphere infusion, arterial reference samples were withdrawn into counting vials under oil from both femoral artery catheters at a rate of 3.66 ml. per minute for 3 minutes with a Harvard pump. This method allows the calculation of cardiac output as well as individual organ flows. Individual organ blood flows were calculated with the arterial samples by solution of the following equation:

$$\text{Organ flow (ml./min.)} = \frac{\text{Total c.p.m. for organ}}{\text{Mean c.p.m. in arterial sample}} \times \text{Withdrawal speed (ml./min.)} \quad (1)$$

Cardiac output (CO) was obtained by the solution of the following equation:

$$\text{CO (ml./min.)} = \frac{\text{Total microspheres infused}}{\text{Mean microspheres in arterial sample}} \times \text{Withdrawal speed (ml./min.)} \quad (2)$$

Samples for arterial blood gases were drawn from the femoral artery before each epinephrine infusion during construction of dose-response curves and also before and after each isotope-labeled microsphere infusion.

The ewes were killed within 24 hours after the second microsphere infusion and dissected for tissue preparation. The uterus, vagina, and cervix were removed *en bloc* and the cervix and vagina then dissected from the uterus. The endometrium and myometrium were separated by sharp and blunt dissection. The efficacy of this method has previuosly been confirmed by histologic examination.[7] The three components of the uterus (cotyledons, endometrium, and myometrium) were then weighed. The following organs were also removed and weighed: brain, mammary gland, lungs, heart, liver, spleen, pancreas, kidneys, ovaries, Fallopian tubes, thyroid gland, and adrenal glands. Representative samples of foreleg skin, mammary gland skin, hindleg muscle, chest wall muscle, and small bowel were also removed and placed directly into counting vials.

Samples were counted in a Nuclear-Chicago automatic dual-channel gamma counter with a 3 inch crystal detector and a wide well. The ^{51}Cr was counted as an emission peak of 0.321 Kev. (window range, 0.271 to 0.371 Kev.) and the ^{141}Ce was counted at a peak of 0.145 Kev. (window range, 0.127 to 0.162 Kev.). Under these conditions 4.5 to 5.5 per cent of the ^{51}Cr counts appeared in the ^{141}Ce window and 0.3 to 0.7 per cent of the ^{141}Ce counts appeared in the ^{51}Cr window. The true counts per minute for each isotope were calculated by the solution of the appropriate simultaneous equation.

Control flows before epinephrine infusion and experimental flows during epinephrine infusion for each organ or tissue studied were calculated. The paired t test was used to determine degrees of significance between control and experimental flows. All mean values are presented as ±1 standard error of the mean (S.E.M.).

Results

Fig. 2 demonstrates the dose-response curves of mean systemic arterial pressure and uterine blood flow to the intravenous infusion of epinephrine. The curves depicting the changes in mean arterial pressure and uterine blood flow are similar in each of the animals studied. There is no significant change in the systemic pressure as the infused dose of epinephrine increases until there occurs a decrease in uterine arterial blood flow of greater than 40 to 50 per cent.

With the use of these dose-response curves, an infusion rate of epinephrine which would lead to the largest decrease in uterine blood flow without alteration in the systemic pressure was chosen for use in the subsequent microsphere studies. The dose employed in these studies ranged from 0.192 to 0.410 μg/min. · kg. with a mean of 0.287 μg/min. · kg. ± 0.034. At this rate of infusion there was a significant change in heart rate, increasing from 102 ± 9.1 to 122 ± 13.4 beats

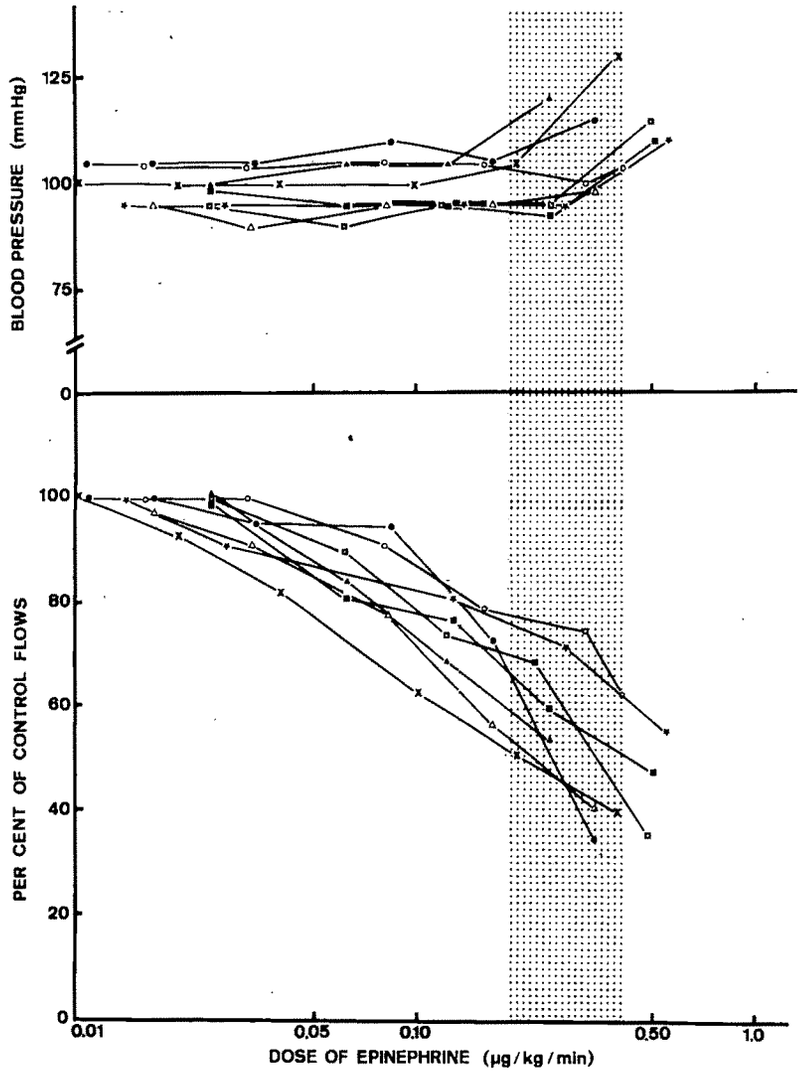


Fig. 2. The responses of uterine blood flow as measured with the flowmeters and mean systemic blood pressure to increasing doses of epinephrine. The shaded area represents the dose range employed in the microsphere portion of the study. (0.2 to 0.4 μg/Kg.·min.). Uterine blood flow is presented as per cent of control flows.

per minute ($p < 0.05$) but no significant alteration in the mean systemic pressure (control of 92.0 mm. Hg ± 3.7 and experimental of 92.3 mm. Hg ± 6.0). A typical response of uterine blood flow to the epinephrine infusion is demonstrated in Fig. 1.

After studying the first two animals and calculating the uterine arterial blood flow from the microsphere data, a discrepancy between this data and the simultaneously observed flowmeter data was noted. This had not been our experience in the past[8] and led to the discovery of a malfunctioning component in the gamma counter. From previous experiments, which permitted the comparison of 61 simultaneous determinations of uterine blood flow by the microsphere and flow probe methods, we were able to construct an ecuation[8] which related the two measurements:

$$\text{Microsphere flow (ml./min.)} = \frac{\text{Flowmeter flow (ml./min.)} - 36 \text{ ml./min.}}{0.86} \quad (3)$$

The uterine arterial flows for the first two animals studied were corrected with the use of Equation (3). As the per cent distribution of the microspheres was known and not affected by the gamma counter malfunction, it was possible to determine the arterial blood flow to each of the uterine tissues. Once the gamma counter was repaired this descrepancy did not recur.

In a third animal we were unable to place the microsphere infusion catheter into the left ventricle.

Table II. The effect of a systemic infusion of epinephrine on total uterine blood flow and blood flow rate to the separate uterine tissues as measured with the microsphere method

	Animal No.							
	1	*2*	*3*	*4*	*5*	*6*	*7*	*Mean ± S.E.M.*
Total uterine flow, without cervix (ml./min.):								
Control	566.7	720.6	1007.0	748.8	1818.0	541.6	793.7	
Experimental	429.8	390.8	656.0	391.7	508.6	419.6	478.5	
Per cent change	−24.2	−45.8	−34.9	−47.7	−55.5	−22.5	−38.6	−38.5 ± 4.6*
Cotyledonary flow (ml./min.):								
Control	379.1	578.3	848.9	535.4	1543.0	443.1	614.5	
Experimental	299.6	339.3	589.7	299.3	739.4	368.1	395.8	
Per cent change	−21.0	−41.3	−34.9	−47.7	−52.1	−22.5	−35.6	−34.5 ± 4.8*
Myometrial flow (ml./min.):								
Control	23.8	19.2	45.7	48.4	53.5	21.7	27.7	
Experimental	20.2	9.7	26.0	27.0	21.3	17.7	19.5	
Per cent change	−15.3	−49.7	−43.2	−44.1	−60.1	−16.3	−29.5	−36.9 ± 6.4*
Endometrial flow (ml./min.):								
Control	163.8	123.1	112.8	165.5	221.6	77.4	151.5	
Experimental	110.0	41.8	40.0	65.4	47.9	33.9	72.2	
Per cent change	−32.8	−66.0	−64.5	−60.5	−78.4	−56.3	−52.3	−58.7 ± 5.3*
Percentage cardiac output to the uterus:								
Control	7.94	9.35	†	†	20.6	‡	14.5	13.1 ± 2.8
Experimental	3.42	4.29			7.38		6.75	5.46 ± 1.9**

*$p < 0.025$.

**The decrease is significant at $p < 0.05$.

†Cardiac output not available, see text.

‡Cardiac output not available, no left ventricular catheter. See text.

Table III. Tissues and organs demonstrating a significant change in arterial blood flow during an epinephrine infusion

Tissue or organ	*Per cent change**	*p*
Skeletal muscle	+434.6 ± 112.4	0.025
Intra-abdominal fat	+180.0 ± 41.5	<0.05
Spleen	+138.9 ± 29.2	<0.01
Pancreas	−27.8 ± 7.6	<0.025
Mammary gland skin	−44.2 ± 10.1	<0.05
Total uterus	−38.5 ± 4.6	<0.025

*Mean ± S.E.M.

The catheter was left in the ascending portion of the thoracic aorta. Because of this, cardiac output and blood flow to those organs served by the ascending aorta were not calculated. Thus, we were able to analyze blood flow to all organs in only four animals, and flows to organs served by the descending aorta for an additional animal. Uterine blood flow data are available for all seven animals studied.

Cardiac output was available in four animals. The mean cardiac output was 7,279 ml. per minute in the control period and 8,937 ml. per minute during the epinephrine infusion. The cardiac output increased 18 to 76 per cent in three animals and decreased 10.5 per cent in the fourth.

Table II shows the effect of epinephrine on myometrial, endometrial, and placental blood flows. When the separate uterine tissues are compared it can be seen that the endometrium exhibited a significantly greater decrease in blood flow (−58.7 per cent ± 5.3) than either the myometrium (−36.9 per cent ± 6.4, $p < 0.025$) or the placental cotyledons (−34.5 per cent ± 4.7 $p < 0.005$). There was no significant difference between the responses seen in the myometrium and placental cotyledons ($p > 0.50$).

In the three ewes carrying singleton pregnancies, the responses to epinephrine in the pregnant and nonpregnant horns of the uterus were compared and no difference was observed. Cervical blood flow was also examined and found to decrease from 6.03 ± 1.06 to 3.94 ± 0.91 ml. per minute but these changes were not statistically significant. No significant changes were seen in ovarian and Fallopian tube flows.

Because of the nature of the animal preparation and the method for measuring blood flow with the isotope-labeled microspheres, it was possible to compare blood flow changes within the uterus with changes of blood flow to other organs and tissues. The results of this comparison are presented in Tables III and IV and Fig. 3. Skeletal muscle obtained from the hindlimb and the chest wall, pooled samples from peritoneal and

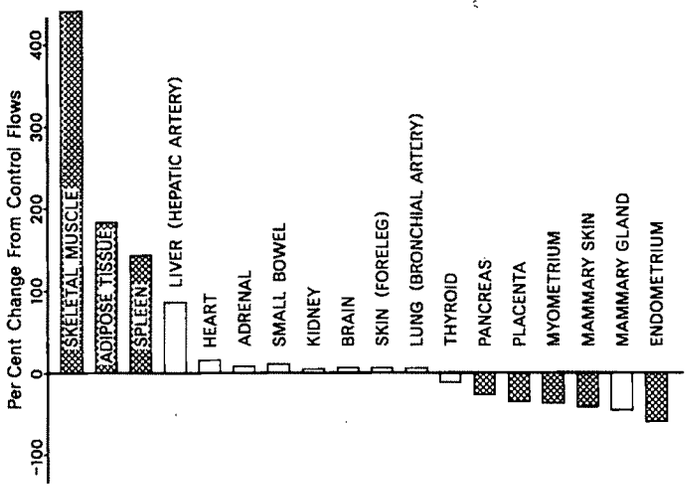


Fig. 3. The per cent change in organ and tissue blood flows during a systemic infusion of epinephrine (0.2 to 0.4 μg/min. · Kg.) in pregnant ewes. The shaded bars are statistically significant at $p < 0.05$.

omental fat, and the spleen demonstrated significant increases in flow, whereas the uterine tissues, skin overlying the mammary gland, and the pancreas showed significant decreases in flow. There was a slight increase in flow to the hind leg skin that was not statistically significant.

The data for organ blood flows in the four ewes in which cardiac output was determined were analyzed in order to determine the change in the distribution of cardiac output that occured during the infusion of epinephrine (Table IV). It should be noted that there was a significant decrease in the percentage of cardiac output going to the kidneys.

The blood flow to the ovine mammary gland was noted to increase with gestational age of the animals in these experiments. Our data demonstrated control mammary blood flow of 15.9 ml. per minute at 85 days of gestation and 228 ml. per minute at 140 days of gestation. In each of the animals studied the infusion of epinephrine consistently caused a decrease in mammary blood flow ranging from 13 to 70 per cent but, because of the large variation in the control flows, the decrease demonstrated was not statistically significant ($0.10 > p > 0.05$). No significant changes were seen in arterial pH or $Paco_2$ during the studies. Values were consistent with those previously recorded.[9]

Comment

The vascular effects of catecholamines have been widely studied with systemic and individual organ infusions in both man and laboratory animals.[10] The new features of the present study are (1) the demonstration of epinephrine-induced vasoconstriction in each component of the vascular bed of the pregnant uterus and (2) the greater sensitivity of the reproductive tissues to the vasoconstrictive properties of epinephrine than the other organs or tissues of the body.

Table IV. Organs demonstrating a significant change in the percentage of cardiac ouput received, as determined in four ewes

Organ	*Per cent cardiac output**		*p†*
	Control	*Experimental*	
Right kidney	5.3 ± 0.3	4.14 ± 0.2	<0.05
Left kidney	5.2 ± 0.5	4.13 ± 0.5	N.S.
Combined kidneys	10.5 ± 0.5	8.27 ± 0.5	0.05
Spleen	3.8 ± 0.4	6.6 ± 0.1	0.005
Pancreas	2.1 ± 0.4	1.3 ± 0.3	<0.025
Total uterus without cervix	13.1 ± 2.8	5.5 ± 1.9	<0.05

*Mean ± S.E.M.

†Paired analysis, Student's t test.

The effect of catecholamines on uterine blood flow was first studied in detail by Robson and Schild.[11] Writing in 1938, they found that epinephrine produced uterine arterial vasoconstriction in both pregnant cats and spayed cats treated with estrone or with estrone and progesterone. The contractile response of the myometrium to catecholamines is known to depend on the hormonal state of the animal.[12] Their results indicated that the response of the uterine vasculature to epinephrine was free of female hormonal influence. In subsequent studies by Ahlquist and Woodbury[2] in pregnant dogs and by Adams and associates[1] in pregnant sheep, the vasoconstrictive effects of epineph-

rine on uterine blood flow were confirmed. Studies on the effect of systemic catecholamine infusions on blood flow to the gravid ovine uterus have been reported by Greiss.[3] In these studies, he reported a 50 per cent reduction in uterine vascular conductance following systemic infusions of greater than 0.5 μg/Kg./min. of epinephrine or norepinephrine. There was no significant difference in response over the period of gestation studied, 112 to 147 days. Our study, which demonstrates in ewes from 85 to 140 days of gestation a 39 per cent reduction in uterine arterial flows during systemic infusions of epinephrine ranging from 0.2 to 0.4 μg/Kg./min., is in good agreement with Greiss' data.

The present study documents that, at the dose levels that were employed, the vasculature of all three uterine tissues (endometrium, myometrium, and placental cotyledons) are sensitive to the vasoconstrictive effects of epinephrine. It was further demonstrated that the response of the endometrial vasculature to epinephrine (a 59 per cent reduction in blood flow) was greater than that noted in either the placental cotyledons (a 35 per cent reduction) or the myometrium (a 37 per cent reduction), whereas the responses in these two tissues were similar. This documentation of a vascular response by all of the uterine tissues to epinephrine suggests the presence of alpha receptors in the arterioles of each of the uterine tissues and presents evidence that these receptor sites remain sensitive to the vasoconstrictive effects of epinephrine during pregnancy.

Arterial blood flow to the mammary gland and to the skin overlying the mammary gland was decreased by the epinephrine infusion (see data). Mammary gland arterial blood flow has been shown to decrease in response to epinephrine.[13, 14] In contrast to the mammary gland skin, skin from the ventral surface of the hind leg did not demonstrate a significant reduction in blood flow. Skin vasculature may vary in its reactivity to systemic catecholamine infusions according to its location.

In the dose range of epinephrine studied here, 0.2 to 0.4 μg/Kg./min., skeletal muscle, intra-abdominal fat, and splenic blood flows were all increased significantly, whereas blood flows to the pancreas were decreased.

The vasodilatory effect of epinephrine on skeletal muscle has been attributed to beta/receptor stimulation. Low doses of epinephrine given intra-arterially into the forearm muscle of man produce a transient sharp rise in the arterial flow of greater than 100 per cent of control, followed by a lower, sustained flow that is greater than 50 per cent of control.[15] These results are supported by data from the present study which demonstrate a striking increase in blood flow to chest wall and hindlimb muscle (>400 per cent of control) in the face of unaltered mean systemic pressure.

Epinephrine has been domonstrated to cause an increase in blood flow to adipose tissue in this study. The phenomenom has been demonstrated in canine subcutaneous adipose tissue infused with epinephrine at a concentration of 0.01 μg per milliliter.[16] In the same study isoproterenol was noted to increase fat blood flow, suggesting that beta-receptor stimulation can lead to vasodilation in adipose tissue.

The splenic vasculature has been widely studied and found to respond to infused catecholamines.[17, 18] Beta-adrenergic stimulation led to increases in splenic blood flow, whereas alpha stimulation decreased flow. Epinephrine was demonstrated in the cat to increase splenic blood flow from 60 to 160 per cent of control flows[17, 18] following doses from 0.1 to 1.0 μg per kilogram. In the pregnant sheep studied here splenic blood flow also increased without a systemic blood pressure increase. It is possible that with the use of higher doses of exogenous epinephrine, splenic vasoconstriction might have occurred.

The arterial bed of the pancreas has been shown by other investigators to possess both alpha and beta receptors,[19] but the response of this organ to catecholamines is by no means settled. We were able to demonstrate a significant reduction in pancreatic blood flow of approximately 28 per cent, occurring 2 to 3 minutes after the initiation of the epinephrine infusion, and not associated with an alteration in systemic arterial pressure.

Arterial blood flow to tissues such as heart, brain, kidney, liver, and bowel has been found to be altered by catecholamines in various studies, but did not demonstrate a significant change following the 0.2 to 0.4 μg/Kg.·min. epinephrine infusion employed here in pregnant sheep. Though no significant change in blood flow to the kidneys was documented during the epinephrine infusion, the per cent cardiac output reaching them was significantly reduced. As the skeletal muscle makes up a large part of the body mass in these animals, it is conceivable that the increase in flow to this tissue (>400 per cent) accounts for both the increase and redistribution of cardiac output.

Epinephrine is of use in various clinical situations in obstetrics, such as an adjuvant for local anesthetic solutions. If epinephrine were inadvertently given in sufficient quantity into the vascular system during a local anesthetic injection, it might have a deleterious effect on uterine blood flow in the pregnant human. Slow intravenous or subcutaneous epinephrine in the human subject has little effect on mean blood pressure,

but does significantly decrease peripheral resistance and increase both cardiac output and heart rate.[10] In the experiments reported here, a dose of epinephrine that produced no significant change in mean systemic blood pressure did significantly increase muscle blood flow, did significantly increase heart rate, and in three of four instances did increase cardiac output. The same dose also led to a 38 per cent reduction in total uterine blood flow in the pregnant ewe. On the basis of these findings, it can be suggested that deleterious effects might occur in a pregnant woman exposed to relatively low arterial concentrations of epinephrine.

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A double-blind investigation into the effects of ritodrine on uterine blood flow during the third trimester of pregnancy

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The object of this double-blind crossover study was to investigate the effect of a β-mimetic agent–ritodrine–administered intravenously at increasing infusion rates on uterine blood flow and the maternal cardiovascular system during the third trimester of normal pregnancies and pregnancies complicated by intrauterine growth retardation and/or hypertension. Uterine blood flow, maternal arterial blood pressure, maternal and fetal heart rate, and uterine activity were simultaneously recorded throughout the study. In normal pregnancies the changes in different parameters during ritodrine and placebo administration were slight. In the "pathologic" pregnancies there was a highly significant increase in maternal heart rate, differential blood pressure, and uterine blood flow during ritodrine administration. The differences in response to ritodrine and placebo were statistically significant in this later group. This study demonstrates increased sensitivity of the maternal cardiovascular system to this β-mimetic agent in certain categories of high-risk pregnancies and possibly an increase in uteroplacental blood flow.

DURING recent years there have been many reports on the use of a beta-mimetic agent, ritodrine, for the treatment of premature labor[1–4] and as a uterus relaxant.[5, 6] In addition, animal studies have suggested a possible effect of β-mimetic drugs on uterine and placental blood flow.[7–9] Sivasambo[3] showed an increased infant weight at the thirty-ninth week of pregnancy, which has not been confirmed by other authors.[10]

The object of this study was to investigate in a double-blind cross-over study the effect of ritodrine, given by the intravenous route in a very low dose, on the uterine blood flow and cardiovascular system during the third trimester of pregnancy.

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Materials and methods

Included in the study were 18 patients in the third trimester of normal pregnancy and 11 patients in the third trimester of pregnancies which were termed "pathologic"—that is, with confirmed intrauterine growth retardation or hypertension. After detailed explanation of the procedures involved and after obtaining consent of the patient to these procedures, the study commenced.

All patients were informed that they were free to drop out of the study at any time without any personal consequences.

Trial procedures

A thermistor probe* was inserted into the anterior lip of the cervix to a depth of 20 mm. and, by utilizing the technique of anenometric thermometry described by Brotanek and associates,[11] increases or decreases in the rate of heat dissipation from the probe tip could be registered. The changes have been shown to reflect changes in blood flow past the probe tip. Local blood

*Captor made by "Institut de physique biologique," Strasbourg University.

flow is one of the most prominent factors involved, but not the only one. Interstitial fluid flows, and probe movement, for example, can modify the indication of blood flow. The placement of the probe 20 mm. deep in the cervix measures, at best, blood flow in cervical tissues. This may not reflect total uterine blood flow and, even more importantly, placental perfusion. The uteroplacental and myometrial vascular beds may show different reactivities.

In one patient, at 38 weeks of gestation with intrauterine growth retardation, the membranes ruptured during insertion of the thermistor probe. The patient went into labor and a healthy 2,200 gram infant was born.

Maternal cardiovascular response. The maternal heart rate was recorded from digital photoplethysmography or from the electrocardiogram. Maternal systolic and diastolic blood pressures were measured indirectly by use of an external sphygmomanometer.

Uterine activity. This was monitored continuously throughout the study by external tocography* and initially in the first two cases by internal tocometry through an open-end fluid-filled catheter.†

In these two patients uterine contractions appeared during the insertion of an intrauterine catheter. After that event the intrauterine pressure recorder was not used in this study.

Fetal heart rate. This was continuously recorded from the phonocardiogram.

Simultaneous recording of all these parameters was done on a Philips polygraph.‡

This study, which was executed under strictly double-blind conditions and on a randomized cross-over basis, consisted, for each patient, of a 4 hour study period divided into five phases. There was an initial phase of 30 minutes during which the various records were allowed to reach a stable baseline. This was followed by a second phase during which three intravenous infusions were given, each of 10 minutes' duration, at a rate of 50, 100, and 150 μg per minute of ritodrine in 5 per cent dextrose, or an equivalent amount of a placebo of identical appearance, via an infusion pump.§

This was followed by a third period of 20 to 60 minutes during which the various recorded parameters were once more permitted to reach a constant baseline. Thereafter, the procedure was repeated according to the cross-over design.

*Hewlett Packard No. 8020A.
†Plasticath No. 8728-27, Plastimed, France.
‡Philips Cardiopan 8, Liechti-Philips A.G., Bern.
§Infors, Basel, Switzerland.

Table I. Excluded cases

Case	*Reason for exclusion*
Two normal cases	Uterine contractions after insertion of transparietal intrauterine catheter for intrauterine pressure recording
One "pathological" case	Rupture of membranes during insertion of thermistor probe in cervix

Table II. Changes in uterine blood flow during administration of ritodrine and placebo

Pregnancy	*Increased flow*	*Decreased or unchanged*	*Uninterpretable*	*N.S.*
Normal:				
R*	2	10	4	N.S.
P	3	10	3	
Pathologic:				
R	7	3	0	P = 0.02; Fisher's exact test, two-sided
P	1	9	0	

*R = Ritodrine; P = placebo.

The study was performed always in the same room with a controlled environmental temperature, by the same personnel, and with the patient in the same posture throughout (semilateral left decubitus).

Results

There were 29 patients included in the study but the results of three were excluded from the analysis. Details of the reasons for exclusion are given in Table I.

Uterine blood flow. Table II shows the uterine blood flow responses for the two patient groups. In the normal pregnancy group no consistent responses were seen to the infusion with either ritodrine or placebo.

In the group of "pathologic pregnancies" uterine blood flow increased in seven of the 10 subjects during ritodrine infusion as compared with one of 10 during infusion with placebo. This difference in response is statistically significant ($p = 0.02$ Fisher's test, two-sided).

Maternal cardiovascular responses.

Maternal heart rate. The maternal heart rate responses of the two groups are shown in Fig. 1. In the normal pregnancies there was no significant difference in the responses to ritodrine and placebo; in the "pathologic pregnancies" significant tachycardia was seen within 20 minutes of commencing ritodrine

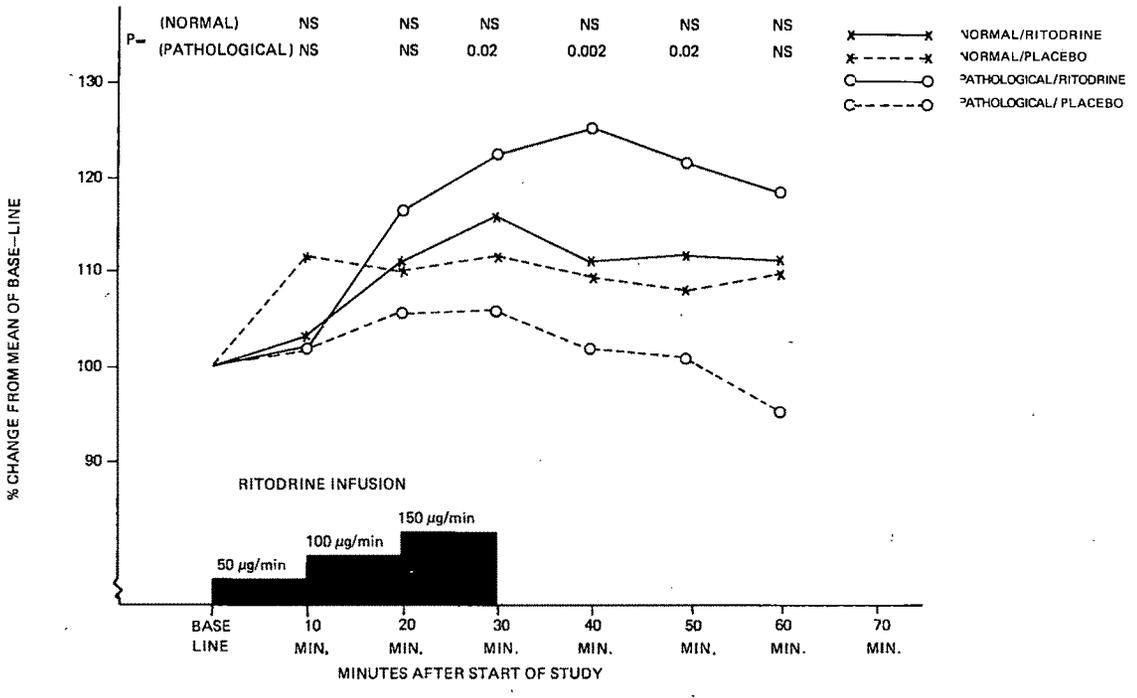


Fig. 1. Changes in maternal heart rate during ritodrine and placebo infusion.

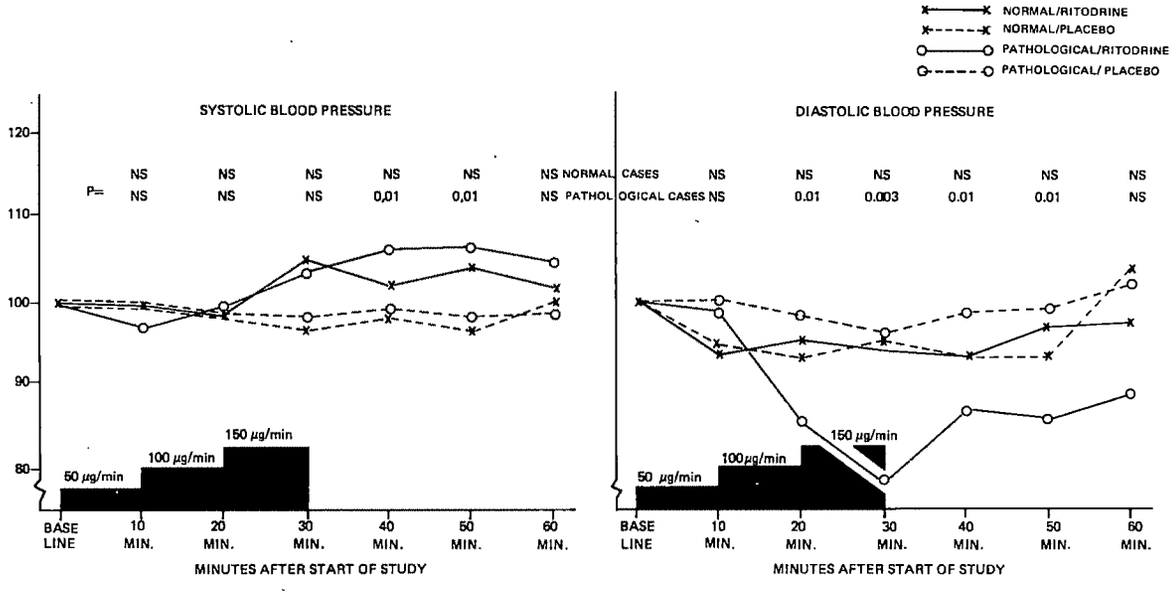


Fig. 2. Effect of ritodrine and placebo on maternal blood pressure.

infusion. This difference was still apparent 20 minutes after stopping the infusion ($p = 0.02$).

Maternal blood pressure. The maternal blood pressure responses are shown in Fig. 2. In the normal pregnancies, there was no significant effect of ritodrine or placebo on diastolic or systolic blood pressure.

In the group of pathologic pregnancies during ritodrine infusion there was a small but significant increase in the systolic blood pressure and a marked decrease in the diastolic pressure. The difference in response to ritodrine and placebo was still apparent at 20 minutes after stopping the infusion ($p = 0.01$). The blood-pressure changes resulted in highly significant effects on the differential blood pressure (pulse pressure), which was increased during ritodrine infusion in the pathologic pregnancies ($p = 0.006$ at 30

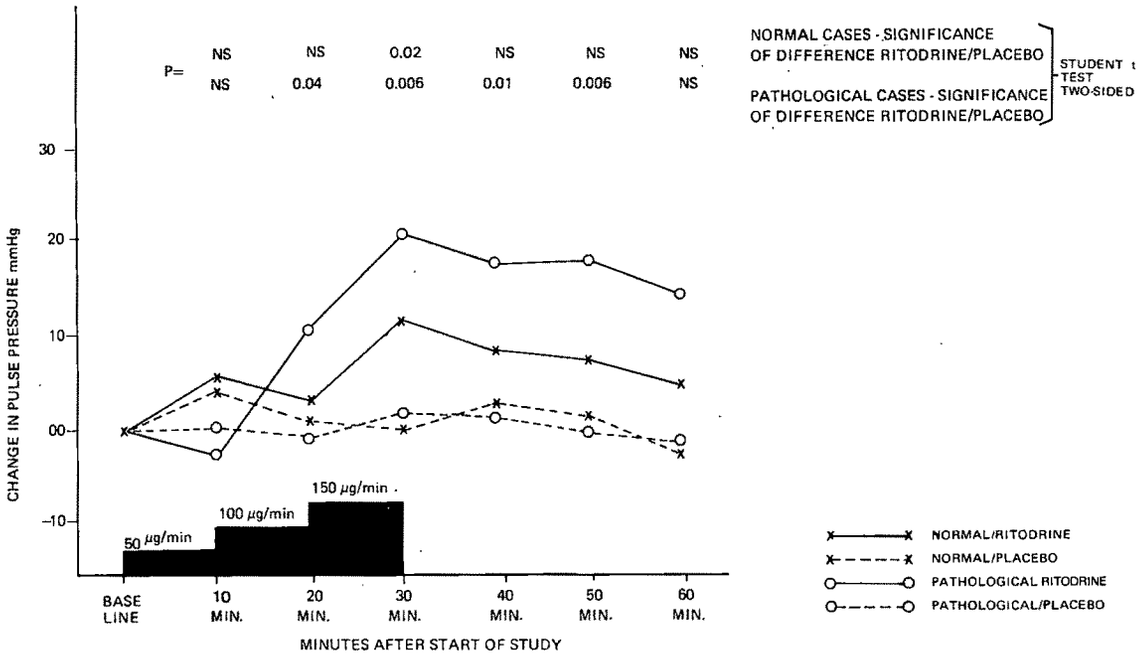


Fig. 3. Changes in pulse pressure during infusion of ritodrine and placebo.

minutes of infusion); in the normal pregnancies the difference in response to ritodrine and placebo was significant only during the last 30 minutes of infusion ($p = 0.02$) (Fig. 3).

Effect on fetal heart rate. In none of the study periods in either group was any significant effect on fetal heart rate observed, at the dosage used.

Uterine activity. With the exception of the two patients who had uterine contractions after the insertion of an intrauterine catheter, in none of the other patients was any effect seen on uterine activity.

Comment

The results of this study suggest that in patients with uncomplicated pregnancies intravenous infusion of ritodrine at the low dose of 150 µg per minute has no effect on uterine blood flow as measured by heat loss from the tip of a heated probe. In pathologic pregnancies, however, beta stimulation appears to result in an increase in uterine blood flow in certain patients, whereas infusion of a placebo in the same patients under the same experimental conditions does so rarely. A further difference in response to beta stimulation is seen in the maternal cardiovascular system. At the infusion rates used in this study the maternal cardiovascular responses in the patients with uncomplicated pregnancies were very slight and with the exception of differential blood pressure at the maximum infusion rate the differences in response to ritodrine or placebo were not statistically significant. In the patients with hypertensive pregnancies are intrauterine growth retardation there was a highly significant difference between the responses to ritodrine and placebo. Despite the low infusion rate of the beta mimetic there was a significant increase in maternal heart rate, a small but significant increase in systolic blood pressure, and a marked fall in diastolic pressure.

The most significant net response was a large increase in the differential blood pressure (pulse pressure). It is possible that the increased heart rate during ritodrine infusion results in increased uterine blood flow.

Bieniarz and associates[12] have demonstrated an increase in cardiac output during infusion of high doses of ritodrine. This study confirms the cardiovascular effects which Bieniarz described but suggests that patients with certain types of high-risk pregnancy may be more sensitive to the beta mimetic in terms of cardiovascular responses and indirectly, perhaps, in uterine blood flow responses.

A number of authors have suggested that there may be an effect on fetal weight as a result of administration of ritodrine during pregnancy. Sivasambo[3] mentioned increased weight for gestational age following ritodrine administration in a controlled trial in premature labor. Seidl and associates[10] compared the infant weights of patients treated with ritodrine and untreated patients. They could find no significant difference between the mean weights at any gestational age;

however, when treatment was started after the thirty-sixth week of pregnancy, the number of babies weighing less than 3,000 grams was larger than when the treatment was started before the thirty-fifth week; nevertheless, this difference was not significant.

Animal studies have demonstrated a highly significant effect on the incidence of intrauterine growth retardation.[13] It is interesting to note in these studies that there was no increase in the incidence of large offspring but a very significant reduction in the incidence of growth-retarded ones.

Janisch and associates[14] have reported studies on placental blood flow using the passage of a technitium-99 bolus through the placental site as an index of change in flow and concluded that beta-mimetic infusion resulted in increased flow in 10 cases and decreased flow in four other pathologic cases.

Wallenburg and associates,[9] using another beta mimetic, suggested an effect of beta-mimetic administration on placental flow in monkeys.

Our results are consistent with those findings.

There is a possibility that ritodrine would be of great interest in the treatment of intrauterine growth retardation.

Controlled clinical trials are still necessary to evaluate whether these changes persist during long-term administration and with other routes of administration.

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Termination of midtrimester pregnancy by serial intramuscular injections of 15(S)-15-methyl-prostaglandin $F_{2\alpha}$

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Midtrimester abortion was successfully induced in 117 of 120 patients with serial intramuscular injections of 15(S)-15-methyl-prostaglandin $F_{2\alpha}$ (15-me-$PGF_{2\alpha}$). The mean abortion time was 14.12 hours, and parous patients aborted in a mean of 12.85 hours—significantly faster than nulliparous patients who aborted in a mean of 15.24 hours. Ninety-four per cent of the 117 successfully induced abortions occurred in less than 24 hours and 46 per cent in less than 12 hours. Uterine activity was monitored and analyzed in nine patients. Uterine response to a single intramuscular injection of 100 μg of 15-me-$PGF_{2\alpha}$ was characterized by the appearance of low-amplitude, high frequency contractions and a rapid increase in baseline intrauterine tonus. A high level of uterine activity, 900 Montevideo Units, was observed within 30 minutes of the first intramuscular injection of 15-me-$PGF_{2\alpha}$. This activity was not maintained and decreased by approximately 30 per cent at the time of the second injection at 1 hour. It was not until 6 hours of 15-me-$PGF_{2\alpha}$ therapy that activity stabilized at approximately 500 Montevideo Units. Even though all patients were premedicated with antiemetic and antidiarrhea agents, 68 of 120 patients experienced gastrointestinal side effects related to the 15-me-$PGF_{2\alpha}$ administration. Vomiting was the most prevalent side effect, occurring in 65 patients, but the episodes were not severe, were well tolerated by the patients and did not necessitate the termination of prostaglandin administration in any of the patients. In this study abortion was successfully induced between weeks 9 to 27 of gestation. It was observed that patients with gestations of 16 weeks or less aborted significantly faster than patients with gestations of 17 weeks or more, which indicates that this method is highly effective in the induction of abortion within the "gray zone", 12 to 16 weeks of gestation.

DURING THE PAST several years there have been a number of reports describing the uterine stimulating and abortifacient effects of naturally occurring prostaglandins. The uterine response to a single injection of these compounds was relatively short, probably due to a rapid enzymatic inactivation. Dehydrogenation occurs at carbon-15 and the resulting prostaglandins possess greatly reduced biologic activity. In order to maintain good uterine activity and achieve abortion, continuous infusion or repeated administration by the intrauterine or vaginal route was required.

Bundy and co-workers[1] reported the synthesis of prostaglandins modified at carbon-15, and that these prostaglandins resisted enzymatic degradation by the enzyme prostaglandin 15-dehydrogenase. The modified prostaglandins 15(S)-15-methyl-prostaglandin E_2 and $F_{2\alpha}$ (15-me-PGE_2 and 15-me-$PGF_{2\alpha}$) were then evaluated for clinical usefulness. Karim and Sharma[2] reported that the 15(S)-15-methyl-prostaglandin E_2 methyl ester and 15(S)-15-methyl-prostaglandin $F_{2\alpha}$ free acid were more potent and had a longer duration of uterine-stimulating action than did PGE_2 and $PGF_{2\alpha}$. The uterine-stimulatory effects of the 15-

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Table I. Patient population by parity and gestational age in the study of intramuscular 15-me-$PGF_{2\alpha}$ for induction of abortion

Parity	*Weeks of gestation*					
	12 or fewer	*13-14*	*15-16*	*17-18*	*19-20*	*21+*
0	5	12	14	12	14	6
1	3	7	7	5	3	1
2	0	4	0	2	1	2
3+	1	6	3	8	4	0

methyl analogue of prostaglandins E_2 and $F_2\alpha$ were confirmed in the rhesus monkey by Kirton and Forbes.[3] Toppozada and co-workers[4] reported that the threshold dose for inducing contractions in midpregnancy with single intravenous doses of 15-me-$PGF_{2\alpha}$ was approximately one tenth of the threshold dose of the naturally occurring $PGF_{2\alpha}$. Intramuscular injection of this 15-methyl analogue in a dose of 1.0 to 1.5 mg. administered to women in the midtrimester induced a marked uterine-stimulatory effect which was sustained from 5 to 7 hours. Karim and associates[5] successfully induced midtrimester abortion by intramuscular injection of 15-me-$PGF_{2\alpha}$. Lauersen and Wilson[6] reported on successful induction of midtrimester abortion in a series of 35 women by serial intramuscular injection of 15-me-$PGF_{2\alpha}$. A preliminary group of five patients in that study were treated with a dose scheduled of 250 μg intramuscularly every 6 hours, but only one patient aborted within 24 hours on that dose schedule. The remaining patients aborted in 30 to 37 hours. The dose schedule for the following 35 patients was then altered to 250 μg initially, followed by 250 μg in 2 hours, and then 500 μg every 2 hours until the time of abortion; the mean abortion time with this dose schedule was 16 hours and 86 per cent of the patients aborted in under 24 hours. It was observed that the appearance of gastrointestinal side effects, which occurred in 90 per cent of the patients, was mostly related to the first dose of 15-me-$PGF_{2\alpha}$. A greatly expanded series has therefore been undertaken in which the initial dose of intramuscular 15-me-$PGF_{2\alpha}$ has been reduced to 100 μg in an attempt to reduce the gastrointestinal side effects of the drug. This study has been designed to determine the abortifacient efficacy of the revised dose schedule and to monitor the effects of abortion induced by intramuscular 15-me-$PGF_{2\alpha}$ on uterine activity, as well as progesterone and cortisol levels.

Materials and methods

A total of 120 healthy women volunteers, ranging in age from 12 to 41 years (mean age, 23 years) and with gestational ages ranging from 9 to 27 weeks (Table I), were included in this study. There were 63 nulliparous patients and 57 multiparous patients in this series. Abortion was induced by serial intramuscular injections of 15(S)-15-methyl-prostaglandin $F_{2\alpha}$* (15-me-$PGF_{2\alpha}$). An initial dose of 100 μg of 15-me-$PGF_{2\alpha}$ was administered intramuscularly, followed in 60 minutes by 250 μg intramuscularly, then 2 hours later by 500 μg intramuscularly. A dose schedule of 500 μg of 15-me-$PGF_{2\alpha}$ was then administered intramuscularly every 2 hours until the time of abortion or a maximum of 24 hours of therapy. If a patient had not aborted within 24 hours, the dose was increased to 750 μg of 15-me-$PGF_{2\alpha}$ intramuscularly every 2 hours until the time of abortion. There were two exceptions in this series to the regular dose schedule. One patient was a 31-year-old woman, para 0, gravida 1, at 26 weeks' gestation, with severe pre-eclampsia, who received 50 μg every 2 hours for a total dose of 200 μg. This patient aborted in less than 7 hours. The second patient, a 28-year-old women, para 1, gravida 2, in the thirteenth week of gestation, developed very strong uterine contractions with the 15-me-$PGF_{2\alpha}$. The dose schedule in this patient was 100 μg, followed in 1 hour by 250 μg and in 2 hours by 250 μg. She aborted in 4.50 hours at a total dose of 600 μg. The dose range in the remaining patients was 1.35 to 11.85 mg. (mean, 3.30 mg.).

In order to minimize gastrointestinal side effects all patients were premedicated and continued on antiemetic and antidiarrhea agents throughout the abortion period. The premedication was 10 mg. of Compazine† (prochlorperazine) and four tablets of Lomotil‡ (each tablet contains diphenoxylate hydrochloride 2.5 mg. and atropine sulfate 0.025 mg.) administered orally 60 minutes prior to the initial injection of 15-me-$PGF_{2\alpha}$. Two tablets of Lomotil were then administered every 2 hours and the Compazine 10 mg. was given intramuscularly every 6 hours until the time of abortion.

A complete blood count, including hematocrit, hemoglobin, white blood cell count, differential, and platelet count, as well as liver function tests, plasma progesterone, and plasma cortisol levels were performed prior to the administration of the 15-me-$PGF_{2\alpha}$, at 8 hours and at 24 hours after initiation of the procedure, in 63 patients. In the remaining 57 patients blood tests, excluding cortisol and progesterone, were

*Supplied by Edward M. Southern, M.D., of the Upjohn Company, Kalamazoo, Michigan.

†Smith, Kline & French Laboratories, Philadelphia, Pennsylvania.

‡G. D. Searle and Company, Chicago, Illinois.

carried out prior to the initiation of the 15-me-$PGF_{2\alpha}$, at 8 hours, and at the time of abortion. Following routine hospital procedure, the patients were given an intravenous infusion of Ringer's lactate and blood was obtained for typing and cross-matching. Vital signs including heart rate, blood pressure, temperature, and respiration were monitored throughout the procedure at frequent intervals.

Uterine activity was monitored in nine patients via an open-end catheter inserted intra-amniotically as previously detailed.[7] The catheter was connected to a pressure transducer (Hewlett Packard, Waltham Division, Model 267 BC, Waltham, Massachusetts) and a dual-channel carrier amplifier recorder (Hewlett Packard, Sanborn Division, Model 321, Waltham, Massachusetts). Spontaneous uterine activity was observed for approximately 20 minutes prior to the prostaglandin administration.

When the fetal and placental tissue were completely expelled from the uterus, or the placenta was removed from the cervical os manually or with sponge forceps without the need for anesthesia, the abortion was defined as being complete. An incomplete abortion was defined as partial expulsion of the products of conception from the uterus, requiring surgical evacuation under general anesthesia for completion.

Results

Mid-trimester abortion was successfully induced in 117 of 120 patients by serial intramuscular injections of 15(S)-15-methyl-prostaglandin $F_{2\alpha}$. The mean abortion time(the interval from the first intramuscular injection to the expulsion of the fetus) was 14.12 hours. Parous patients aborted somewhat faster(mean,12.85 hours) than the nulliparous(mean, 15.24 hours) and this difference was statistically significant ($p < 0.05$) (Table II). In this series of the 117 successfully aborted patients 95 per cent aborted in less than 24 hours, and 46 per cent aborted in less than 12 hours (Fig. 1). Two patients, who aborted in more than 24 hours, were former heroin addicts and at the time of the abortion were on methadone maintenance, receiving 80 to 90 mg. of methadone per day. Both patients were extremely tense and anxious, presenting difficult management problems. Included in this series, there was a patient with twin pregnancy of 20 weeks' gestation. The patient responded well to the medication and aborted in under 10 hours without complication.

There were three trials that were declared failures in this series. The first failure occurred in a 41-year-old multiparous patient, with a uterine myoma, in the twentieth week of gestation. This patient developed

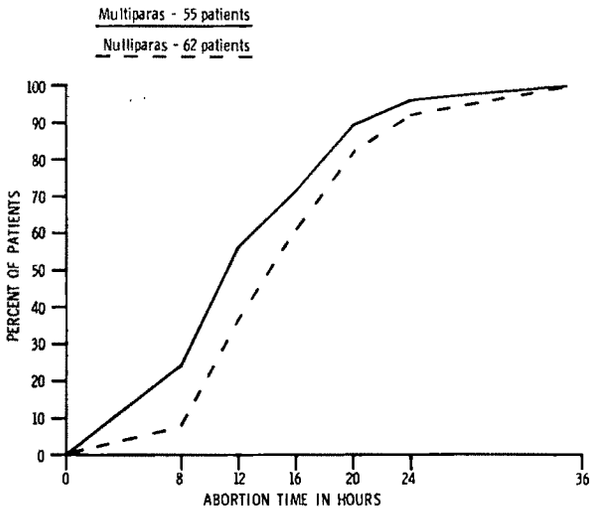


Fig. 1. The cumulative abortion rate versus time for the 117 successful midtrimester abortions induced by serial intramuscular injections of 15-me-$PGF_{2\alpha}$ in mulliparous and multiparous patients.

Table II. Intramuscular 15-me-$PGF_{2\alpha}$ for midtrimester abortion

Patients	*No. of patients*	*Abortion time (hr.)*
Nulliparas	62	15.24 ± 6.17
Multiparas	55	12.85 ± 5.80
Total	117*	14.12 ± 6.09

*The three failures of this technique have been excluded from these calculations.

adequate uterine contractions and the membranes ruptured at 20 hours, but she did not abort despite high uterine activity. The patient subsequently developed endometritis and the response to prostaglandin diminished. She was started on antibiotics and a surgical completion was eventually performed without difficulty since the cervix was soft and well dilated and the products of conception were removed in parts. The patient was subsequently discharged in good condition.

The second failure occurred in a 30-year-old woman, para 1, in the fifteenth week of gestation. This patient did not abort within 24 hours despite high uterine activity; a sonogram was thereafter obtained which demonstrated a 10 by 10 cm. cervical fibroid that obstructed the cervical canal. A surgical completion was subsequently performed without difficulty and the patient was discharged in good condition.

The third failure took place in a 19-year-old nulliparous patient in the twentieth week of gestation. This patient did not abort despite strong uterine activity. A review of the family history revealed that the

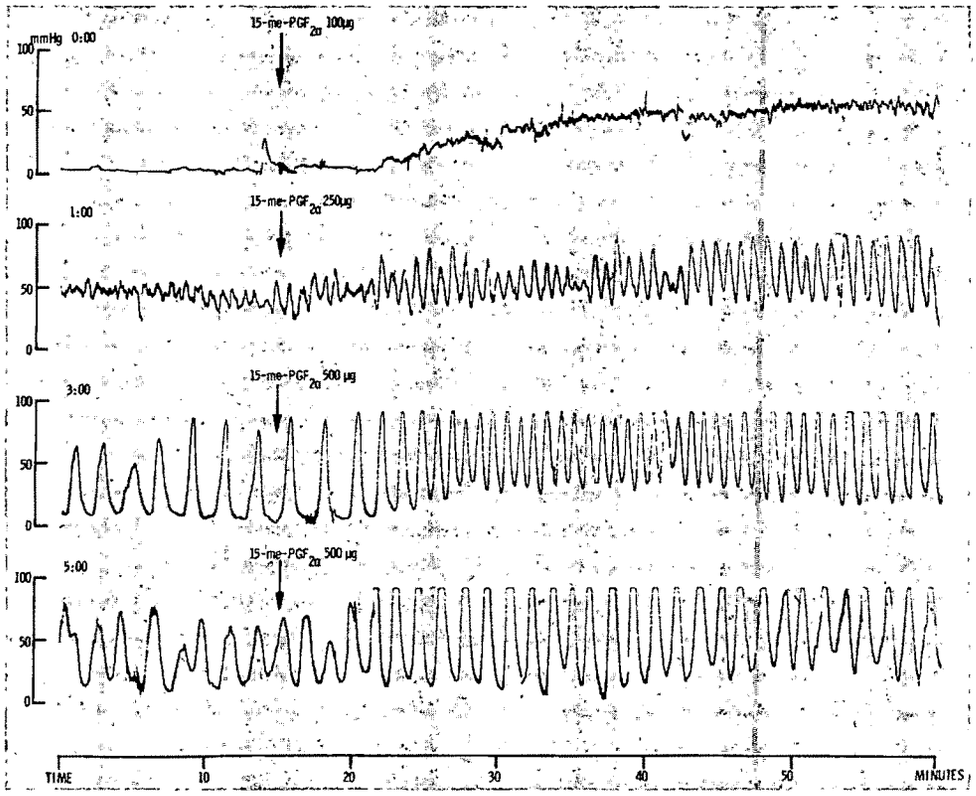


Fig. 2. Tracing of intra-amniotic pressure showing the effects of midtrimester abortion induced by serial intramuscular injections of 15-me-$PGF_{2\alpha}$ on uterine activity in a single patient. The tracing is in 60 minute segments and illustrates the uterine effects of four intramuscular injections of 15-me-$PGF_{2\alpha}$.

Table III. Intramuscular 15-me-$PGF_{2\alpha}$ for midtrimester abortion

Weeks of gestation	*No. of patients*	*Abortion time (hr.)*
12 weeks or fewer	9	14.61 ± 7.87
13	9	9.84 ± 4.94
14	20	11.76 ± 3.72
15	12*	12.63 ± 5.59
16	11	13.73 ± 4.43
17	8	11.87 ± 2.78
18	19	15.60 ± 6.04
19	10	16.93 ± 5.70
20	10*	14.32 ± 6.23
21+	9	20.86 ± 8.27

*The three failures of this technique have been excluded from these calculations.

patient's sister had a double uterus and sonogram performed on this patient then revealed a double uterus with the pregnancy in the left uterine horn. When the patient did not abort within 27 hours she was taken to the operation suite for surgical completion. A stenotic ring was felt at the level of the internal os of the left uterine horn, which could have explained the patient's failure to abort with intramuscular 15-me-$PGF_{2\alpha}$. The patient was subsequently discharged in good condition.

Intramuscular 15-me-$PGF_{2\alpha}$ was an effective abortifacient throughout the gestational periods tested in this series (Table III). It was observed that patients with lower gestational ages, 9 to 16 weeks, aborted significantly faster (mean, 12.42 hours) than patients with gestational ages of 17 weeks and higher (mean, 15.96 hours) ($p < 0.01$).

The uterine response to the initial intramuscular injection of 100 μg of 15-me-$PGF_{2\alpha}$ was characterized by the appearance of low-amplitude, high-frequency contractions and a rapid rise in baseline intrauterine tonus. Subsequent injections produced further increments in uterine activity (Fig. 2). The initial 100 μg of 15-me-$PGF_{2\alpha}$ intramuscular injection produced a mean of 905 Montevideo Units (M.U.) (average amplitude × number of contractions in 10 minutes) within 30 minutes in the nine patients monitored. Uterine activity then declined to a mean of 612 M.U. at 1 hour and at this time a subsequent dose of 150 μg of 15-me-$PGF_{2\alpha}$ increased uterine activity to a mean of 923 M.U. within 30 minutes. Uterine activity declined to a mean of 386 M.U. at 3 hours of therapy when a

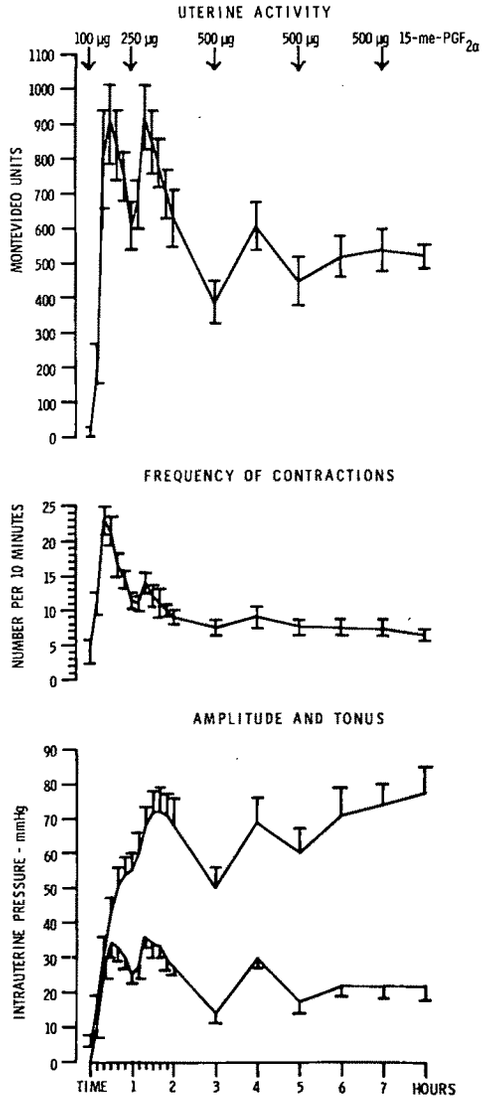


Fig. 3. The effect of serial intramuscular injections of 15-me-PGF$_{2\alpha}$ on uterine activity, calculated in Montevideo Units (average amplitude × number of contractions over a 10 minute period), frequency of contractions, amplitude of contractions, and baseline intrauterine tonus, illustrated by mean and standard error of the mean over an 8 hour period in nine patients.

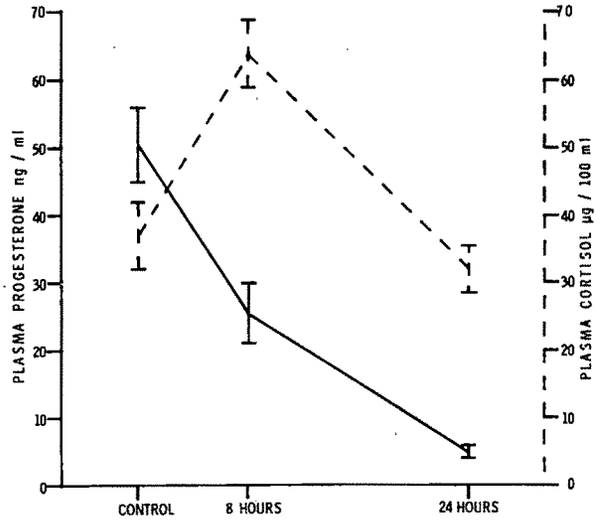


Fig. 4. The effect of midtrimester abortion induced by serial intramuscular injections of 15-me-PGF$_{2\alpha}$ on plasma progesterone and plasma cortisol levels in 63 patients. The results are illustrated by mean and standard error of the mean.

Table IV. Side effects of intramuscular 15-me-PGF$_{2\alpha}$ for midtrimester abortion

	Patients	*Episodes*
Gastrointestinal side effects	68 (58%)	240
Nausea	17	35
Vomiting	65	183
Diarrhea	8	22
Temperature elevation 2° F.	30	

Table V. Complications of intramuscular 15-me-PGF$_{2\alpha}$ for midtrimester abortion

Complications	*No. of patients*
Failure of technique	3
Incomplete abortion	2
Endometritis (failure of technique)	1
Placenta removed by sponge forceps	16
Placenta removed manually	14
Blood loss 300 ml. and over	3

third dose of 15-me-PGF$_{2\alpha}$ was administered and again activity increased. At about 6 hours into the prostaglandin therapy uterine activity stabilized around a mean of 500 M.U. (Fig. 3). Frequency of contractions peaked at 20 minutes after the first injection of 15-me-PGF$_{2\alpha}$ at a mean of 23 contractions per 10 minutes. The frequency of contractions then subsided, and the second injection of 15-me-PGF$_{2\alpha}$ produced a second increase in frequency to 14 contractions per 10 minutes. The frequency then slowly declined to stabilize at mean of seven contractions per 10 minutes throughout the abortion period. The amplitude of contractions increased steadily after the first two injections of prostaglandin to a mean maximum of 74 mm. Hg within 30 minutes of the second injection. Amplitude declined to a mean of 51 mm. Hg at 3 hours, immediately prior to the third dose of prostaglandin, and then rose; declined at 5 hours, and then steadily rose throughout the abortion period to a mean of 78 mm. Hg at 8 hours. Intrauterine baseline tonus increased rapidly to a maximum mean of 32 mm. Hg 30 minutes after the first 15-me-PGF$_{2\alpha}$ injection. The

Table VI. The effect of midtrimester abortion induced by intramuscular 15-me-$PGF_{2\alpha}$ on hematocrit, white blood cells, and platelet count

	Control	*8 hours*	*At abortion*	*No. of patients*
Hematocrit (%)	34.1 ± 3.2	34.8 ± 3.4	33.6 ± 3.8	57
White blood cells ($\times 10^3$)	8.8 ± 2.6	13.0 ± 3.5	13.3 ± 3.8	57
Platelet count	268 ± 68	274 ± 67	277 ± 77	57
			24 hours	
Hematocrit (%)	34.2 ± 2.9	36.2 ± 3.1	33.8 ± 2.9	63
White blood cells ($\times 10^3$)	8.8 ± 2.4	12.7 ± 3.5	11.0 ± 2.8	63
Platelet count	263 ± 63	270 ± 64	257 ± 65	63

subsequent prostaglandin injection produced a mean intrauterine baseline tonus of 36 mm. Hg. Like amplitude, tonus decreased at 3 hours, peaked again at 4 hours, and then stabilized around 22 mm. Hg at 6 hours.

Of the 120 patients included in this series, 68 experienced gastrointestinal side effects related to the administration of intramuscular 15-me-$PGF_{2\alpha}$. Vomiting was the most prevalent side effect, experienced by 65 patients, and 54 patients had more than one episode of vomiting. Nausea was experienced by 17 patients, with 10 patients having more than one episode. Diarrhea was the least common gastrointestinal side effect, and only eight patients (7 per cent) experienced diarrhea. There was a temperature elevation of more than 2° F. in 30 patients but this temperature elevation was small and only seven patients had a temperature of 102° or higher. Temperatures returned to normal levels in all patients within 4 to 6 hours after the last intramuscular injection of 15-me-$PGF_{2\alpha}$ (Table IV). There were three failures of technique in this series, as previously detailed, and two incomplete abortions. One patient who failed to abort with intramuscular 15-me-$PGF_{2\alpha}$ developed endometritis. The placenta was removed by sponge forceps in 16 patients and manually in 14 patients.

There was an estimated blood loss of 300 ml. or more in three patients but there was no need for transfusion for any patient (Table V). The mean estimated blood loss in this series was 82.81 ± 69.71 ml. (range, 30 to 450 ml.). Mean hematocrit and platelet levels reflected the minimal blood loss observed in this series of abortions. There were no statistically significant changes in mean hematocrit and platelet levels throughout the procedure. The mean white blood count (WBC) increased significantly at 8 hours, with a further increase in mean WBC at the time of abortion. There was some decrease at 24 hours, but the 'mean WBC had not returned to preinjection levels at this time (Table VI).

Plasma progestrone levels decreased significantly in the 63 patients tested 8 hours after the first intramuscular injection of 15-me-$PGF_{2\alpha}$ to 50 per cent of the control level, and at 24 hours the plasma progesterone was 9 per cent of the control level. Plasma cortisol increased significantly at 8 hours after the first injection and decreased to preinjection levels by 24 hours (Fig. 4).

Comment

Abortion was successfully induced in 117 of 120 patients from the ninth to the twenty-seventh weeks of gestation with serial intramuscular injections of 15-me-$PGF_{2\alpha}$ with a mean time of 14.12 hours. The initial dose of 15-me-$PGF_{2\alpha}$ was 100 μg, followed by 250 μg in 60 minutes, and then 500 μg every 2 hours until abortion, as compared to our previous study[6] where the dose schedule was 250 μg every 2 hours for the first 2 hours, followed by 500 μg every 2 hours until the time of abortion. The mean abortion time in this latest, expanded study compares favorable with the abortion time in the previous study, with a mean time of 15.98 hours, which indicates that a high initial dose of intramuscular 15-me-$PGF_{2\alpha}$ is not necessary for a successful outcome. The incidence of gastrointestinal side effects on the new dose schedule was only 56 per cent, as compared to 90 per cent in the previous study, where high initial doses were employed. It therefore appears that the initiation of prostaglandin-induced abortion with a low initial dose helps to reduce gastrointestinal side effects of the drug without increasing the over-all abortion time. Once a patient's sensitivity to the drug was established the patient tolerated high doses of the drug given repeatedly without gastrointestinal disturbances.

A high level of uterine activity was observed within 30 minutes of the first intramuscular injection of 100 μg of 15-me-$PGF_{2\alpha}$; however, this level of activity was not maintained and it decreased by approximately 30 per cent at the time of the second injection at 1 hour. Activity increased rapidly immediately after the second injection, but within 2 hours activity had again

decreased by 60 per cent, and it was not until 6 hours of 15-me-$PGF_{2\alpha}$ therapy that the activity stabilized. In a previous study of the abortifacient effectiveness of serial intramuscular injections of 15-me-PGE_2[8] a high level of uterine activity (mean, 500 Montevideo Units) was observed within 40 minutes of a 5 μg intramuscular injection of 15-me-PGE_2. There was no rapid decrease in activity with 15-me-PGE_2, indicating that this drug has a more sustained effect on the uterus than 15-me-$PGF_{2\alpha}$, and the 15-me-PGE_2 appeared to be a minimum of 20 times more potent than the 15-me-$PGF_{2\alpha}$. In another previous study with extraovular administration of the naturally occurring $PGF_{2\alpha}$ for midtrimester abortion,[9] 5 mg. of the drug were required to achieve a similar level of uterine activity as obtained with 100 μg of 15-me-$PGF_{2\alpha}$, indicating that the 15-me-$PGF_{2\alpha}$ is approximately 50 times more potent than the naturally occurring $PGF_{2\alpha}$, although a direct comparison cannot be drawn due to the differences in the methods of administration.

The most prevalent side effect observed in this series of abortions induced by intramuscular 15-me-$PGF_{2\alpha}$ was vomiting, which occurred in 65 of the 120 patients. The vomiting was transient in nature; in none of the cases was it necessary to terminate prostaglandin therapy due to this side effect. Diarrhea was the least common gastrointestinal side effect observed; it occurred only in 7 per cent of the patients. When abortion was induced with 15-me-PGE_2[8] the incidence of gastrointestinal side effects was low—in only five of 30 patients. There was a temperature elevation in only 30 of the 120 patients treated with intramuscular 15-me-$PGF_{2\alpha}$ as opposed to a temperature elevation in 29 of 30 patients treated with intramuscular 15-me-PGE_2. These results seem to indicate a difference between the actions of 15-me-$PGF_{2\alpha}$ and 15-me-PE_2. The E_2 compound may have a more specific effect on temperature regulation, whereas the $F_{2\alpha}$ compounds may have a more specific effect on gastrointestinal motility.

It has been established from a number of studies that the incidence of changes in coagulation factors consistent with DIC is higher in saline-induced abortion than the incidence encountered when midtrimester abortion is induced with intra-amniotic instillation of $PGF_{2\alpha}$[10–12]. Although there have been no studies on the clotting factors when abortions have been induced by 15-me-$PGF_{2\alpha}$, we found no incidence of severe hemorrhage in the total of 160 patients treated with 15-me-$PGF_{2\alpha}$ in the two series we have performed. Evaluation of hematocrit and platelet count showed no significant change during the abortion and postabortion periods, which might indicate that the incidence of DIC in midtrimester abortion induced by 15-me-$PGF_{2\alpha}$ is low, similar to that found in abortion induced with naturally occurring prostaglandin by the intra-amniotic route.

Midtrimester abortion induced by intramuscular injections of 15-me-$PGF_{2\alpha}$ was well accepted by the patients and by the nursing staff of the abortion unit. The patient was spared the painful, time-consuming, and potentially hazardous procedure of amniocentesis required for instillation of hypertonic saline or prostaglandin. The intramuscular injection of 15-me-$PGF_{2\alpha}$ caused very little pain, and no local reaction at the injection site was observed. We believe after conducting this series that the initial three doses of 15-me-$PGF_{2\alpha}$ of 100, 250, and 500 μg should be administered by a physician in order to determine the patient's sensitivity to the drug and that, once the patient's sensitivity has been established, it is safe for subsequent injections to be administered by an alert and informed nurse.

Four patients in this study presented with a history of bronchial asthma and prophylactic aminophylline suppositories were administered, they had no indication of asthma or even minimal asthma attacts during the 15-me-$PGF_{2\alpha}$ therapy. In five patients the fetus was expelled but the placenta was retained; intramuscular 15-me-$PGF_{2\alpha}$ therapy was continued and the placenta was subsequently passed. Intramuscular 15-me-$PGF_{2\alpha}$ might have clinical usefulness in cases of incomplete abortion.

Several patients in this series had originally been admitted to the hospital for suction abortion. Upon examination they were found to be beyond the gestational age acceptable for this operative procedure. Induction of abortion with intramuscular injections of 15-me-$PGF_{2\alpha}$ was very successful and these patients could be discharged within 24 hours. Patients with gestational ages of 16 weeks or less aborted significantly faster than patients with gestational ages of 17 weeks and higher. The abortifacient effectiveness of intramuscular injections of 15-me-$PGF_{2\alpha}$ in early gestations contrasts with our findings in earlier studies on the effect of $PGF_{2\alpha}$ administered via intra-amniotic instillation[7] and continuous extraovular infusion.[9] In these previous studies the $PGF_{2\alpha}$ was found to be a more efficient abortifacient during later gestations of 18 weeks and higher. 15-me-$PGF_{2\alpha}$ administered by intramuscular injection may prove to be a valuable clinical tool, particularly in the induction of abortion during the so-called "gray zone," gestations between the twelfth and sixteenth weeks. From the study of uterine activity it appeared that administration of 15-me-$PGF_{2\alpha}$ was necessary every 2 hours to initially

maintain effective uterine activity, but it is possible that after 6 hours of administration, when uterine activity had stabilized, the frequency of injection could be decreased to every 4 to 6 hours without substantially affecting the outcome of the abortion.

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Effectiveness of certified nurse-midwives

A prospective evaluation study

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A prospective evaluation study of the effectiveness of the services of certified nurse-midwives demonstrated that in a hospital setting the care of low-risk maternity patients provided by nurse-midwives was as effective as that provided by house staff physicians. A total of 438 low-risk maternity patients were studied. Selected outcomes pertaining to the prenatal period, labor and delivery, and early infancy demonstrated, with two exceptions, no significant differences. The two exceptions were: (1) overcompliance with appointment attendance was more common among the nurse-midwifery group of patients; (2) a higher rate of forceps delivery was reported among the house staff group of patients.

THE INCREASING UTILIZATION of nurses in expanded roles and its potential for meeting the health needs of a large sector of the population have generated the need for the evaluation of the services they provide. The evaluation of one such extended role, that of the certified nurse-midwife (CNM), constitutes the focus of this report.

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Recent concern over relatively high maternal and infant mortality and morbidity rates in the nation and the awareness of a large pool of expectant mothers who receive little or no prenatal, intrapartal, and postpartal care have prompted an increase in the training and employment of nurse-midwives. A number of evaluation studies of nurse-midwifery services have been carried out.

Most of them are of a "before and after" nature, in which the study population serves as its own control, with the inherent assumptions and weaknesses of that method. In 1969, Montgomery[1] published a study which showed the effectiveness of a nurse-midwifery "nurse obstetric assistant" service in a rural California county hospital. Following the introduction of the service for normal patients, the proportion of patients receiving first trimester care doubled from five per cent and among primiparous patients increased from 30 to 40 per cent. An increase in the frequency and regularity of prenatal visits was found, and, during the last six months of the study period, 78 per cent of deliveries in the hospital were attended by a nurse obstetric assistant. Over a three-year period from 1959 (before the project's inception) to 1961 (after it began), there was a decline in prematurity rates from 11 per

cent to 6.4 per cent and in neonatal mortality rate from 23.9 per 1,000 live births in 1959 to 10.3 per 1,000 live births in 1961.

Similar positive results were shown by Levy and co-workers.[2] The effectiveness of the nurse-midwifery program was shown not only by an increased utilization of services, but also by a resultant decline in rates of prematurity and neonatal deaths. These indications of effectiveness were further corroborated by a significant reduction in prenatal attendance and a rise in neonatal death and prematurity in the hospital following cessation of the CNM program.

Similar beneficial results were shown in a private team practice in Ohio[3] and in a prepaid group practice.[4]

One evaluation study[5] did attempt to apply an experimental design through the random allocation of patients to the CNM or the resident staff services of two hospitals. This further documented the effectiveness of nurse-midwives. Unfortunately, the potentially biased nature of the determination of "uncomplicated" status performed by the nurse-midwife and the absence of statistical tests lend caution to ready acceptance of the findings.

This report describes a prospective study to evaluate the effectiveness of nurse-midwives in a university hospital clinic setting in Jackson, Mississippi.

Study setting

The service described in this paper has operated at the University of Mississippi Medical Center, since 1969. The nurse-midwifery care team consists of CNM's, nurse-midwife assistants, obstetricians, and residents in obstetrics and gynecology who serve as consultants, provide back-up care, and assume temporary or permanent management of complicated cases. The same house staff, obstetricians, residents, and medical students provide care to all other maternity patients. All patients are delivered in the hospital.

The nurse-midwifery service provides prenatal, intrapartal, postpartal, family planning, and infant health supervision services.

Both CNM and house staff (HS) service programs are designed to provide clinical practice for students. Patients do not see the same provider consistently in either service.

The HS team provides comprehensive prenatal, intrapartal, and early postpartal care for the patients.

Purpose of the study

The study was intended to evaluate the effectiveness of care provided to obstetric patients by nurse-midwifery and house staff services. Variables selected to measure the effectiveness of the two services included the following prenatal, intrapartal, and immediate postpartal outcomes in the mother and infant. (1) prenatal outcomes—prenatal visit score (actual number of visits ÷ expected number of visits × 100; anemia (hematocrit < 30 per cent); incidence of hypertension rise of >30 mm. systolic or > 15 mm. diastolic pressure; incidence of diabetes (physician's diagnosis based upon urine test, fasting blood sugar, and glucose tolerance test); incidence of indicators of toxemia (physician's diagnosis based upon proteinuria, edema, average weight gain > two pounds per week, rise of blood pressure >30 mm. systolic or >15 mm. diastolic); incidence of urinary tract infection (as diagnosed by culture and sensitivity >100,000 colonies or more than "slight growth" or on microscopy at least two of the following: ten to 15 red cells, 15 to 20 white cells, moderate to heavy bacteruria); rates of investigation for urinary tract infection (performance of urinalysis, culture, sensitivity, or testuria, plus evaluation of clinical findings); rates of investigation for diabetes (performance of urinalysis, fasting blood sugar and glucose tolerance test); (2) maternal outcomes of labor and delivery (obtained from the record of delivery)—length of labor, abnormalities of labor, hours membranes ruptured before delivery, type of delivery, episiotomy, amniotomy, abnormalities of puerperium; (3) infant outcomes (evaluated by the attending neonatologist)—gestational age, weight, length, head circumference, apgar score, fetal distress (Presence of meconium, tachycardia or bradycardia, or fetal acisosis), newborn condition (presence or absence of congenital abnormalities or birth injury or aspiration).

Sample and methods

This prospective study followed two groups of pregnant women from registration for prenatal care through labor, delivery, and the immediate postpartal period. The study population consisted of a sample of patients who registered for care at the University Medical Center's prenatal clinic. At the Medical Center, patients are cared for either by certified nurse-midwives or the house staff of the Center. Patients from both services are delivered at the University Medical Center hospital.

The study samples were selected from patients who registered at these clinics for prenatal care between October 1, 1972, and April 30, 1973, and who met the criteria of eligibility for the nurse-midwifery service, described below.

On admission, a clerk obtained the following data through questions and observation: the number of

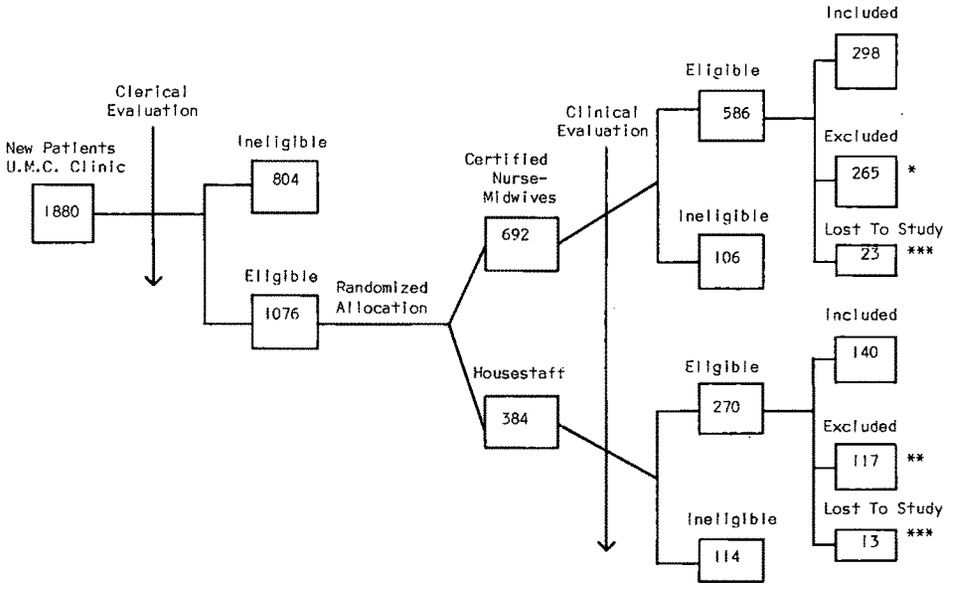


Fig. 1. Selection of patients into study. *Forty-four delivered prior to January, 1972; 221 delivered after December, 1972. **Of these, 29 delivered prior to January, 1972; 88 delivered after December, 1972. ***Delivered elsewhere, moved, or not pregnant.

pregnancies; history of previous cesarean section; diabetes mellitus in the patient; edema and/or varicosities. In the obstetric clinic, urine specimens, blood pressure, height, and weight were determined and the results recorded.

On the basis of these data, initial screening was carried out by the clinic clerk. Patients were excluded if any of the following characteristics were recorded: (1) blood pressure—systolic over 140 mm. or diastolic over 90 mm.; (2) weight above 180 pounds; (3) history of eight or more pregnancies; (4) history of diabetes mellitus; (5) history of cesarian section; (6) history of varicosities or edema.

Patients who requested either house staff or nurse-midwifery services were not included in the study. Those eligible after this "clerical" evaluation were then randomly allocated to nurse-midwifery or house staff service. Patients were allocated to a service according to a computer generated random sequence and implemented by the clerk. The ratio of nurse-midwifery patients to house staff patients was variously 1:1 or 2:1 as the case load of patients for the nurse-midwifery service varied. In the allocated service, a physical examination was provided with initial laboratory test values added to the clinical record.

As a result of this "clinical" evaluation, a further subset of ineligibles was omitted from the study if any of the following characteristics were present: (1) Rh sensitivity; (2) toxemia; (3) multiple gestation; (4) medical history of diabetes mellitus, chronic hypertension, chronic renal disease, severe varicosities of vagina, vulva, or legs, or other severe medical problems; (5) significant bony or soft tissue abnormalities of the pelvis.

Fig. 1 shows the samples in the selection of Medical Center patients. Of the 1,880 new patients admitted during the study period, 804 (43 per cent) were found to be ineligible on clerical evaluation and a further 220 (12 per cent) on clinical evaluation. Thus, 1,024 (55 per cent) of the new patients were deemed ineligible for nurse-midwifery service by set criteria.

Despite efforts to ensure administration of standardized selection criteria, a statistically significantly larger proportion of HS patients (30 per cent) than CNM patients (15 per cent) were eliminated from the study by the clinical evaluation. The possibility exists that an unknown selective factor was operative in the determination of eligibility and may have resulted in some consistent differences between the study groups.

Data were extracted from medical records by coders trained and supervised by a medical records research assistant. By checking against the medical records, quality checks of the data processing were carried out by a nurse on every tenth code sheet and on any questionable codes. Where appropriate, an obstetrician or neonatologist was consulted for clarification.

This report presents the findings with respect to the 438 women who were eligible for study who attended the Medical Center and were delivered there from January 1 to December 31, 1972. Of these, 298 were

Table I. Prenatal visit score by type of service

Prenatal Score	*Nurse-midwifery Service (%)†*	*House staff Service (%)‡*	*Significance*
1-75	24.5	38.6	
76-100	48.0	48.6	$p < 0.0005$
>100	27.5	12.9	
Mean Score	94	80	

*Actual visits as a proportion of expected visits.
†298 Patients.
‡140 Patients.

Table II. Rates of overcompliance* by investigation for urinary tract infection (UTI) by type of service

UTI investigation	*Nurse-midwifery service (%)†*	*House staff service (%)‡*	*Significance*
Yes	25.9	7.7	Not statistically significant
No	30.7	17.3	

*Actual visits as proportion of expected >100 per cent.
†298 Patients.
‡140 Patients.

Table III. Rates of overcompliance* by investigation for diabetes by type of service

Diabetes investigation	*Nurse-midwifery service (%)†*	*House staff service (%)‡*	*Significance*
Yes	30.4	18.9	Not statistically significant
No	25.4	10.7	

*Actual visits as proportion of expected > 100 per cent.
†298 Patients.
‡140 Patients.

nurse-midwifery patients and 140 were house staff patients.

In all comparisons, the chi-square test and t test were applied for categorical and continuous variables, respectively, with the use of a five per cent confidence level as an indicator of statistical significance.

Results

Comparability of the groups. In assessing the comparability of the two service study groups, it was found that there were no significant differences between the groups with respect to age, race, marital status, gravidity, gestation at registration, initial hematocrit, or systolic blood pressure. The mean age was 20.4 years among the CNM patients and 21.7 for the HS patients. The majority of the patients in both groups was black (78.9 per cent in the CNM group and 77.1 per cent in the HS group). Fifty-nine per cent of the CNM group and 64.3 percent of the HS patients were married. Although patients met the low-income criteria for admission to the clinic program, no data were available to compare the groups with respect to socioeconomic status or education. Slightly over 50 per cent of the patients in each group were primigravida, and the average gestation at registration was 26 weeks in each group. The prevalence of anemia at registration (hematocrit less than 30 per cent) was four per cent among CNM patients and five per cent among HS patients. There were no differences between the groups in histories of previous premature births or abortions or in the interval since the last pregnancy. A small but statistically significant discrepancy between the two groups in the distributions at the lower levels of diastolic blood pressure was considered unimportant as a determinant of future outcomes. It was concluded, therefore, that the two patient groups were comparable with respect to relevant and available prenatal characteristics.

Prenatal outcomes. There were no significant differences between the groups during pregnancy in the incidence of excess weight gain, edema, hypertension, urinary tract infection, and diabetes.

About 60 per cent of the patients manifested one or more indicators of toxemia of pregnancy.

Diabetes and urinary tract infections occurred in about one per cent of the subjects.

Nurse-midwifery patients had significantly higher prenatal visit scores than did house staff patients (Table I).

On the average, CNM patients kept 94 per cent of the expected prenatal visits, and the HS patients kept 80 per cent. A higher proportion of CNM patients (27.5 per cent) made more visits than scheduled compared with the HS patients (12.9).

This finding was not explained by a difference in health problems which were found to be equally distributed in the two groups. However, statistically significant differences were found between CNM and HS groups in the rates of investigation for urinary tract infection (UTI) and for diabetes. The CNM's carried out one or more tests for UTI in 90 per cent of their patients compared to 65 per cent by the house staff. Similarly, a greater proportion of CNM patients (43.3 per cent) was investigated for diabetes compared with 27.9 per cent investigated by the house staff. Significantly more overcompliance among CNM patients than among HS patients occurred, regardless of investigation for either UTI or diabetes (Tables II and III).

Table IV. Type of delivery among all patients—Primigravida and multigravida—by type of service

Type of delivery	*All patients (%)*		*Primigravida (%)*		*Multigravida (%)*	
	CNM (298)	*HS (140)*	*CNM (158)*	*HS (71)*	*CNM (140)*	*HS (69)*
Spontaneous	82.6	62.1*	72.8	35.2*	93.6	89.9
Low forceps	9.7	29.3*	17.1	52.1*	1.4	5.8
Mid forceps	2.3	2.9	3.8	4.2	0.7	1.4
High forceps	1.0	1.4	1.3	0	0.7	2.9
Breech	0.7	0	0.6	0	0.7	0
Cesarean section	3.7	4.3	4.4	8.5	2.9	0

Numbers in parentheses are numbers of patients.
*Statistically significant, $p < 0.05$.

Outcomes of labor and delivery. A significantly greater proportion of CNM patients (82.6 per cent) than HS patients (62.1 per cent) had spontaneous deliveries (Table IV). This difference was explained mainly by the rate of low forceps deliveries which was three times higher in the HS patients than in the CNM patients. This excess of low forceps deliveries was found among both primigravida and multigravida.

Low forceps deliveries were still significantly more frequent in the HS patients, even among women without two conditions which indicate forceps deliveries—namely a second stage of labor longer than 60 minutes, or the delivery of a low–birth weight infant (Table V). This suggests that physicians are more likely to perform forceps deliveries for reasons other than the medical indications of prolonged second stage of labor or low birth weight.

There were no significant differences between the groups with respect to the length of the first and third stages of labor, abnormalities of labor, course of labor, the number of hours membranes ruptured before delivery, lacerations, episiotomies, and abnormalities of the puerperium. A significant difference in the duration of the second stage of labor for those with spontaneous deliveries was found among multigravid patients, whereas the HS patients had an average duration of the second stage of labor of 29 minutes compared with an average of 18 minutes among CNM patients. No such difference was found for primigravidas.

Infant outcomes. There were no differences between the two groups with respect to gestational age, newborn condition, or infant length.

While the rate of low–birth weight babies (≤2,500 grams) was higher (9.1 per cent) in the CNM group than in the HS group (five per cent), this difference was not statistically significant. Similarly, there was no significant difference in the mean birth weights of the two groups (3,128 and 3,172 grams, respectively).

Indications of fetal distress (abnormal heart rate and/or the presence of meconium) were reported more often in patients of the HS group than in those of the CNM group. The difference was almost entirely due to the greater proportion of HS group babies with meconium. These differences, while not statistically significant, were demonstrated mainly in primigravida who had spontaneous deliveries.

Table V. Type of delivery by service (excluding low birth weight and second stage labor > one hour)

Type of delivery	*Nurse-midwifery service** (%)	*House staff service†* (%)
Spontaneous	86.0	66.1‡
Low forceps	7.4	27.8‡
Mid forceps	1.7	1.7
Other	4.8	4.3

*229 Patients.
†115 Patients.
‡Statistically significant, $p < 0.00001$.

There were no differences between the CNM and HS groups in the one-minute and five-minute Apgar scores, applying a cutoff point of seven as the minimal acceptable score.[6] Three infants in the CNM group and one in the HS group were stillborn and there was one neonatal death within each group. These numbers were considered too small for statistical analysis.

Comment

It appears that in the hospital setting, prenatal, intrapartal, and postpartal care provided by certified nurse-midwives with physician consultant back-up produces health outcomes equivalent to those of the traditional physician service. Some differences were found.

A number of interpretations which bear upon the evaluation of the services may explain the over-compliance of the certified nurse-midwifery patients. The certified nurse-midwives may be applying a greater number of expected routine visits for their

patients, suggesting that the usual routine number is inadequate to provide good care. Extra visits may be scheduled by the nurse-midwives for additional educational services or the care of social needs. On the other hand, overcompliance may suggest uncertainty of the nurse-midwife, necessitating more frequent patient visits than routinely indicated. Further detailed study will be needed to more correctly interpret this finding.

The rate of forceps deliveries among house staff patients may reflect differences between the services in their respective practices for normal deliveries. The application of forceps may be viewed as a more routine procedure for physicians than for nurse-midwives who do not carry out the procedure themselves. In addition, forceps deliveries may be performed by house staff, when not medically indicated, for teaching purposes.

A number of possible explanations for the higher rate of premature babies among the CNM patients exists. Although standard criteria were administered for the inclusion of subjects into the study, it was pointed out previously that the proportion of house staff patients eliminated from the study at the stage of clinical evaluation was twice that of the nurse-midwifery group. If some form of selectivity was applied here, the house staff group may have been initially a healthier population and, for this reason, experienced a lower rate of low birth weight. This can be confirmed only by a follow-up of those patients eliminated from analysis by the study criteria.

It was not possible to obtain data to test the comparability of the groups with respect to other variables known to be associated with low birth weight. Among others, these include cigarette smoking in mother and father and education of mother and father.

The incidence of low birth weight as well as other unfavorable findings in this low-risk population suggests a need to explore the determinants of these outcomes in similar select groups. The predictors of obstetric complications in a clinically low-risk population may differ from the standard indicators which have been established for high-risk groups. Such a finding would have significant implications for the delivery of obstetric services by nurse-midwives whose care is confined to the low-risk patient.

In summary, the findings of the study warrant the acceptance of certified nurse-midwives for the care of the clinically low-risk maternity patient.

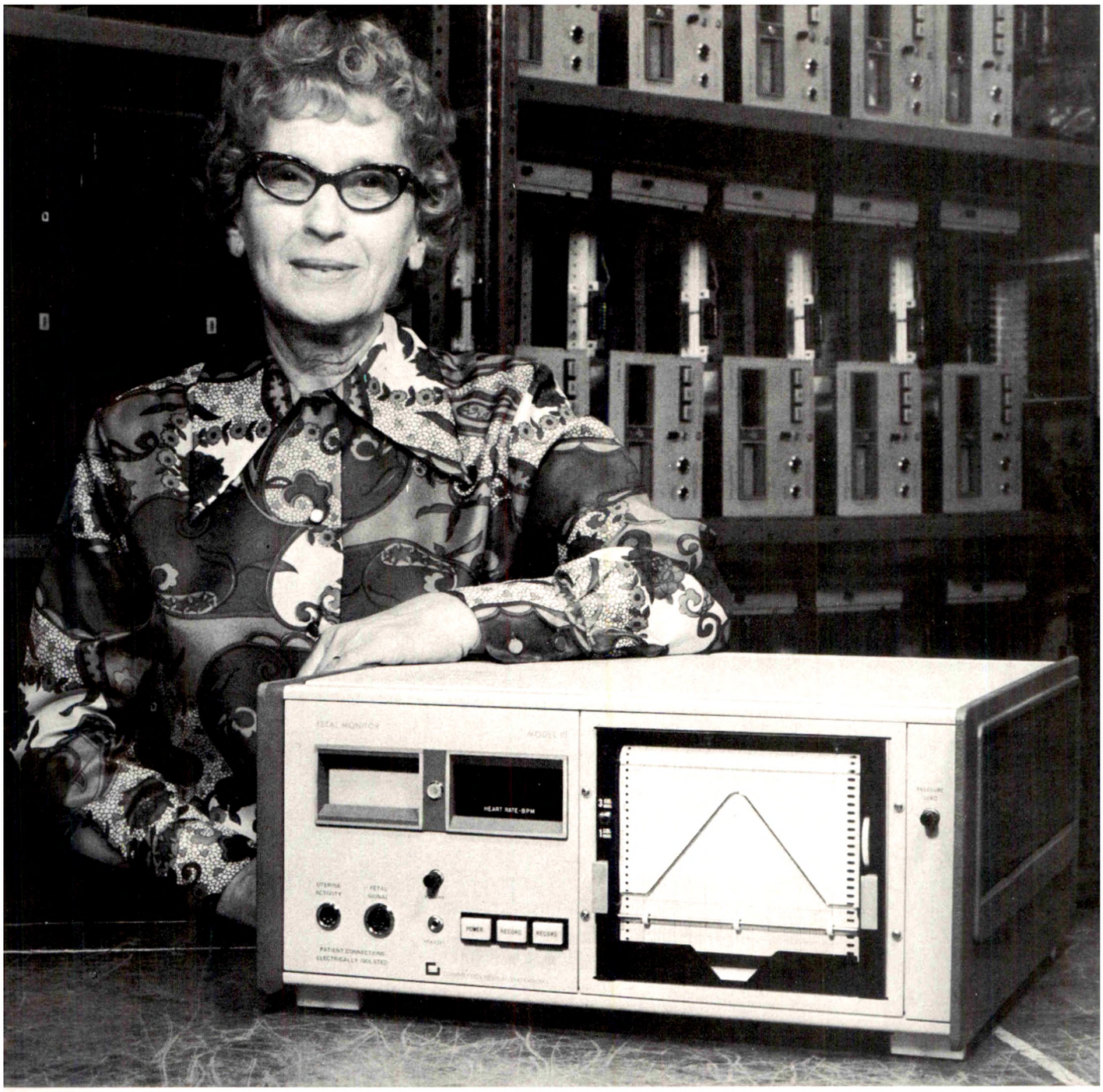
Further evaluation studies of the extension of the role of the nurse-midwife to dealing with high-risk prenatal patients will define the clinical limitations of a successful nurse-midwifery service.

The expanded use of nurse-midwives in the hospital setting has profound implications for the delivery of health services to the maternity population. As care of the normal patient is assumed by the nurse-midwives, physicians will be free to concentrate on high-risk cases. Nurse-midwives may begin to meet the demand for family-centered maternity care. Furthermore, the characteristics and quality of care administered by the nurse-midwife may be especially responsive to certain needs of the population. Finally, nurse-midwives may facilitate a more logical distribution of maternal and infant care providers in that certified nurse-midwives might manage the low-risk aspects of care for all patients, leaving physicians free to attend to the high-risk elements of care.

The authors wish to acknowledge the assistance of Jerry E. Parish and Minta Uzodinma, C.N.M.

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Special note: Oral contraceptives have been marketed in the United States since 1960. Reported pregnancy rates vary from product to product. The effectiveness of the sequential products appears to be somewhat lower than that of the combination products. Both types provide almost completely effective contraception.

An increased risk of thromboembolic disease associated with the use of hormonal contraceptives has now been shown in studies conducted in both Great Britain and the United States. Other risks, such as those of elevated blood pressure and reduced tolerance to carbohydrates, have been reported and appropriate tests should be conducted to monitor these during oral contraceptive therapy. Liver disease has also been reported, and the physician should be alert to its earliest manifestations.

Long-term administration of both natural and synthetic estrogens in subprimate animal species in multiples of the human dose increases the frequency for some animal carcinomas. These data cannot be transposed directly to man. The possible carcinogenicity due to the estrogens can neither be affirmed nor refuted at this time. Close clinical surveillance of all women taking oral contraceptives must be continued.

Indication: Contraception.

Contraindications: 1. Thrombophlebitis, thromboembolic disorders, cerebral vascular disease, or a past history of these conditions. 2. Markedly impaired liver function. 3. Known or suspected carcinoma of the breast. 4. Known or suspected estrogen-dependent neoplasia. 5. Undiagnosed abnormal genital bleeding. 6. Known or suspected pregnancy.

Warnings: 1. The physician should be alert to the earliest manifestations of thrombotic and thromboembolic disorders, thrombophlebitis, cerebrovascular disorders including hemorrhage, pulmonary embolism and retinal thrombosis. Should any of these occur or be suspected, the drug should be discontinued immediately.

Retrospective studies of morbidity and mortality in Great Britain and studies of morbidity in the United States have shown a statistically significant association between thrombophlebitis, pulmonary embolism and cerebral vascular disease, occlusive or hemorrhagic, and the use of oral contraceptives. There have been three principal studies in Great Britain[1-3] leading to these conclusions and three in this country.[4-7] The estimate of the relative risk of thromboembolism in the study by Vessey and Doll[3] was about sevenfold, while the United States studies found relative risks of 4.4 to 11, meaning that the users are several times as likely to undergo thromboembolic disease without evident cause as non-users.

In May, 1974, the Royal College of General Practitioners in England[8] issued an interim report of its continuing large-scale prospective study comparing a user group to a non-user group. This study in its interim analysis states, "A statistically significant higher rate of reporting of cerebrovascular accidents in Takers is evident, but the numbers are too small to justify an estimation of the degree of risk." The study also reported a higher incidence of superficial and deep vein thrombosis in users as compared to non-users. The risk of superficial and deep vein thrombosis was reported to be lower in women using 50 mcg estrogen preparations.

The Sartwell study[4] indicated that the risk did not persist after discontinuation of administration. Both the Sartwell and the Royal College studies indicated that the degree of risk was not associated with duration of treatment.

In a collaborative American study[5,6] of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the relative risk of thrombotic stroke was 4.1 to 9.5 times greater in users than in non-users. A comparable estimate for hemorrhagic stroke was 2.0.

None of the American studies was designed to evaluate a difference between products. However, the Sartwell study[4] suggested that there might be an increased risk of thromboembolic disease in users of sequential products.

Other retrospective studies[9,10] have reported an increased risk of post-surgery thromboembolic complications in oral contraceptive users. It has been recommended that therapy be discontinued at least one month prior to elective surgery.

2. Discontinue oral contraceptive medication if there is: gradual or sudden partial or complete loss of vision; proptosis or diplopia; onset or aggravation of migraine or development of headache of a new pattern which is recurrent, persistent or severe; papilledema; or any evidence of retinal vascular lesions.

3. Fetal abnormalities have been reported to occur in the offspring of women who have taken progestogens and/or estrogens during pregnancy.[11,12] The safety of ORTHO-NOVUM1/80□21 or ORTHO-NOVUM 1/80□28 in pregnancy has not been demonstrated. Pregnancy should be ruled out before initiating or continuing the contraceptive regimen. Pregnancy should always be considered if withdrawal bleeding does not occur.

4. A small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The long-range effect to the nursing infant cannot be determined at this time.

5. Hepatic lesions (adenomas, hepatomas, hamartomas, regenerating nodules, etc.), occasionally fatal, have been reported in women on oral contraceptives. Such lesions may present as an abdominal mass or with the signs and symptoms of an acute abdomen. These lesions should be considered if the patient has abdominal pain or evidence of intra-abdominal bleeding. This has been reported in short-term as well as long-term users of oral contraceptives.

Precautions: 1. A thorough history and physical examination should be performed before prescribing oral contraceptives and periodically during their administration and should include special reference to breasts and pelvic organs, including Papanicolaou smear since estrogens have been known to produce tumors, some of them malignant, in five species of subprimate animals. 2. Endocrine and possibly liver function tests may be affected by treatment with ORTHO-NOVUM 1/80□21 or ORTHO-NOVUM 1/80□28. Therefore, if such tests are abnormal in a patient taking ORTHO-NOVUM 1/80□21 or ORTHO-NOVUM 1/80□28, it is recommended that they be repeated after the drug has been withdrawn for two months. 3. Under the influence of estrogen-progestogen preparations, pre-existing uterine fibromyomata may increase in size. 4. Because these agents may cause some degree of fluid retention, conditions which might be influenced by this factor, such as epilepsy, migraine, asthma, cardiac or renal dysfunction, require careful observation. 5. In breakthrough bleeding, and in all cases of irregular bleeding per vaginam, nonfunctional causes should be borne in mind. In undiagnosed bleeding per vaginam, adequate diagnostic measures are indicated. 6. Patients with a history of psychic depression should be carefully observed and the drug discontinued if the depression recurs to a serious degree. 7. Any possible influence of prolonged ORTHO-NOVUM 1/80□21 or ORTHO-NOVUM 1/80□28 therapy on pituitary, ovarian, adrenal, hepatic or uterine function awaits further study. 8. A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. The mechanism of this decrease is obscure. For this reason, diabetic patients should be carefully observed while receiving ORTHO-NOVUM 1/80□21 or ORTHO-NOVUM 1/80□28 therapy. 9. The age of the patient constitutes no absolute limiting factor, although treatment with ORTHO-NOVUM 1/80□21 or ORTHO-NOVUM 1/80□28 may mask the onset of the climacteric. 10. The pathologist should be advised of ORTHO-NOVUM 1/80□21 or ORTHO-NOVUM 1/80□28 therapy when relevant specimens are submitted. 11. Susceptible women may experience an increase in blood pressure following administration of contraceptive steroids. 12. Cholestatic jaundice has been reported in users of oral contraceptives. If this occurs ORTHO-NOVUM 1/80□21 or ORTHO-NOVUM 1/80□28 should be discontinued. This condition is more likely to occur in patients who have experienced cholestatic jaundice of pregnancy. Patients with a history of cholestatic jaundice of pregnancy should be carefully observed during ORTHO-NOVUM1/80□21 or ORTHO-NOVUM 1/80□28 therapy.

Adverse reactions observed in patients receiving oral contraceptives: A statistically significant association has been demonstrated between use of oral contraceptives and the following serious adverse reactions: thrombophlebitis, pulmonary embolism, cerebral thrombosis and hemorrhage, gallbladder disease.

Although available evidence is suggestive of an association, such a relationship has been neither confirmed nor refuted for the following serious adverse reactions: neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis, hepatic lesions with or without intra-abdominal bleeding.

The following adverse reactions are known to occur in patients receiving oral contraceptives: nausea, vomiting, gastrointestinal symptoms (such as abdominal cramps and bloating), breakthrough bleeding, spotting, change in menstrual flow, amenorrhea during and after treatment, edema, chloasma or melasma, which may persist, cholestatic jaundice, migraine, rash (allergic), mental depression, change in weight (increase or decrease), breast changes: tenderness, enlargement and secretion, changes in cervical erosion and cervical secretions, suppression of lactation when given immediately post-partum, rise in blood pressure in susceptible individuals.

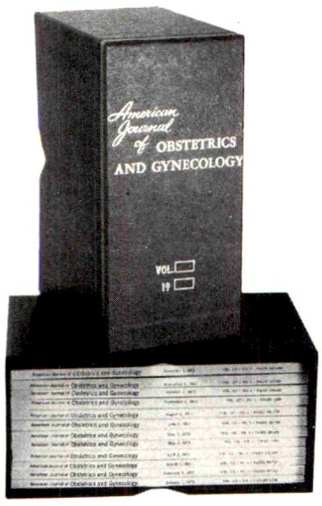
Although the following adverse reactions have been reported in users of oral contraceptives, an association has been neither confirmed nor refuted: anovulation post-treatment, which tends to occur more frequently in women with a history of menstrual disorders, premenstrual-like syndrome, changes in libido, changes in appetite, cystitis-like syndrome, headache, intolerance to contact lenses, nervousness, dizziness, fatigue, backache, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruptions, itching, vaginitis.

The following laboratory results may be altered by the use of oral contraceptives: hepatic function—increased sulfobromophthalein retention and other tests; coagulation tests—increase in prothrombin, Factors VII, VIII, IX and X; decrease in anti-thrombin III; increase in platelet aggregability; thyroid function—increase in PBI, and butanol extractable protein-bound iodine and decrease in T^3 uptake values; metyrapone test; pregnanediol determination.

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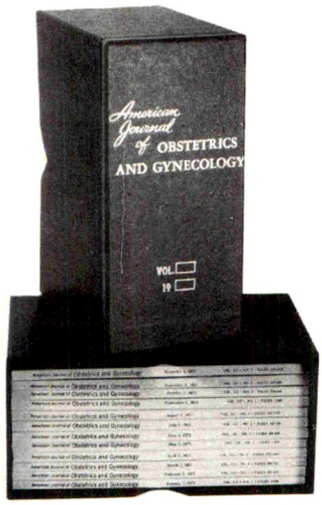
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Abnormal uterine bleeding: progesterone replacement... to oppose unopposed estrogen

Estrogen replacement therapy... a frequent cause of abnormal uterine bleeding

Administration of estrogen for the relief of menopausal symptoms is a frequent cause of irregular bleeding or spotting during the menopause, and the most common cause of uterine bleeding after the menopause. Bleeding may occur when long-term administration of estrogen causes overstimulation of the endometrium.

It cannot be assumed, however, that uterine bleeding in a woman on estrogen replacement therapy is due solely to the exogenous estrogen. The existence of a pathologic condition—benign or malignant—should always be considered, thoroughly explored, and ruled out. It is generally agreed that any woman taking estrogen who complains of abnormal uterine bleeding should undergo a careful physical examination including a Pap test, followed by curettage of the uterine cavity.

A close look at the influence of estrogen and progesterone on the endometrium

In the normal proliferative endometrium (left), the epithelium contains rather flat, nonciliated cells with small microvilli, and scattered, uniform ciliated cells. Regrowth of the surface epithelial cells proceeds vigorously throughout the proliferative phase of the cycle. (X2250)

Under the continued and unopposed influence of estrogen (center), there is a striking increase in the number of ciliated cells and in the abundance and size of the microvillous processes on the nonciliated cells. (X1600)

During the normal secretory phase (right), the presence of progesterone causes the secretory cells to become more complex, and the microvilli to increase in number. Scattered droplets are visible on the surface of some of the nonciliated cells and are attached to the tips of some microvilli. (X2500)



Scanning electron micrographs courtesy of Ralph M. Richart, M.D.

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overa replacement oppose unopposed estrogen

ıen the unopposed action of exogenous estro- ı is responsible for abnormal uterine eding in the menopausal or postmenopausal ient, administration of Provera® edroxyprogesterone acetate), 5 mg - 10 mg ly for 5-10 days, may be all that eeded to control or regulate bleeding. vera, an orally active derivative of gesterone, transforms the estrogen-primed lometrium into a secretory endometrium. eding and rapid shedding of the lometrium occur following cessation of gestin therapy.

ecautions and ntraindications

ıvera appears to be devoid of significant rogenic activity; however, andro- ıic effects have been noted with the drug.

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To control abnormal uterine bleeding in the absence of organic pathology

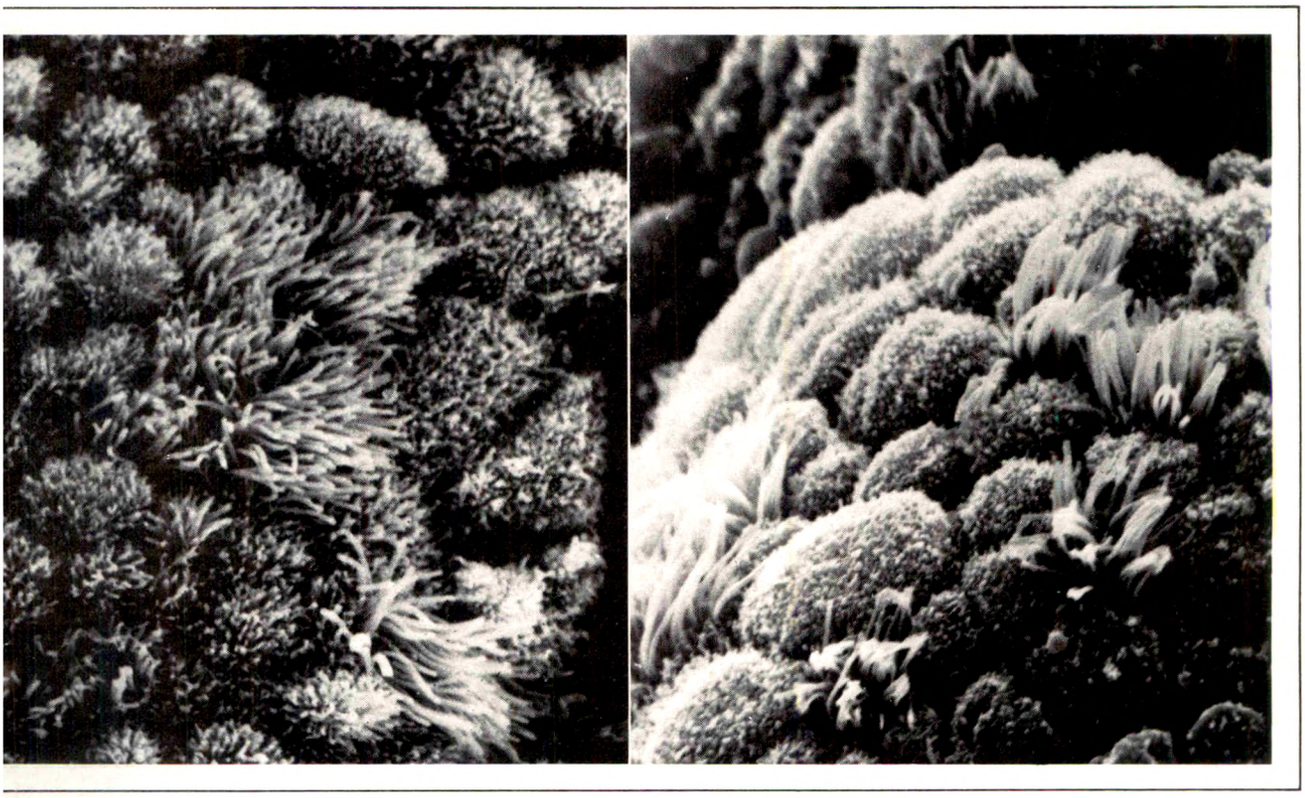
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September, 1974 J-4113-4



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Indications: Provera Tablets are indicated in secondary amenorrhea and abnormal uterine bleeding due to hormonal imbalance in the absence of organic pathology such as fibroids or uterine cancer.

Contraindications: Thrombophlebitis, thromboembolic disorders, cerebral apoplexy or patients with a past history of these conditions. Liver dysfunction or disease. Known or suspected malignancy of breast or genital organs. Undiagnosed vaginal bleeding. Missed abortion. Known sensitivity to medroxyprogesterone acetate.

Warnings: 1. Immediately discontinue administration should any of the following thrombotic disorders occur or be suspected: thrombophlebitis, cerebrovascular disorders, pulmonary embolism, retinal thrombosis. **2.** Beagle dogs treated with medroxyprogesterone acetate developed mammary nodules, some of which were malignant. Although nodules occasionally appeared in control animals, they were intermittent in nature, whereas the nodules in the drug treated animals were larger, more numerous, persistent, and there were some breast malignancies with metastases. Their significance with respect to humans has not been established. **3.** Discontinue medication pending examination if there is sudden partial or complete loss of vision, or if there is a sudden onset of proptosis, diplopia or migraine. If examination reveals papilledema or retinal vascular lesions, medication should be withdrawn. **4.** Detectable amounts of progestin have been identified in the milk of mothers receiving the drug. The effect of this on the nursing infant has not been determined. **5.** Usage in pregnancy is not recommended because of data indicating a possible association between administration of progestins early in pregnancy and congenital heart defects in the offspring. **6.** Three major studies in Great Britain and one in this country have shown a statistically significant association between thrombophlebitis, pulmonary embolism, cerebral thrombosis and embolism and the use of oral contraceptives. It has been estimated that users are several times as likely to undergo thromboembolic disease without evident cause as nonusers. The American study indicated that the risk did not persist after discontinuation and it was not enhanced by long continued administration.

Precautions: A pretreatment physical exam should include special reference to breast and pelvic organs and a Papanicolaou smear. This drug may cause some degree of fluid retention; therefore, observe carefully patients with conditions influenced by fluid retention such as epilepsy, migraine, asthma, and cardiac or renal dysfunction. In breakthrough bleeding, and other irregular bleeding per vaginum bear in mind nonfunctional causes. Perform adequate diagnostic measures in cases of undiagnosed vaginal bleeding. Advise pathologist of therapy when submitting relevant specimens. Carefully observe patients with history of psychic depression and discontinue drug if serious degree of depression recurs. Any possible influence of prolonged therapy on pituitary, ovarian, adrenal, hepatic, or uterine function awaits further study. Decreased glucose tolerance has been observed in a small percentage of patients on estrogen progestin combinations, therefore, carefully observe diabetic patients receiving progestin therapy. Age constitutes no absolute limiting factor, although onset of climacteric may be masked. Because of the occasional occurrence of thrombotic disorders (thrombophlebitis, pulmonary embolism, retinal thrombosis, and cerebrovascular disorders) in patients taking estrogen-progestin combinations and since the mechanism is obscure, the physician should be alert to the earliest manifestation of these disorders.

Adverse reactions: The following have been reported: breast tenderness or galactorrhea in a few instances; an inferential association between the administration of progestins early in pregnancy and the occurrence of congenital malformations (see also Warnings); occasional sensitivity reactions including pruritus, urticaria, edema and generalized rash; acne, alopecia and hirsutism in a few patients; thromboembolic phenomena including thrombophlebitis and pulmonary embolism.

The following adverse reactions have been observed in women taking progestins including medroxyprogesterone acetate: breakthrough bleeding; spotting, change in menstrual flow; amenorrhea; edema, increase or decrease in weight; changes in cervical erosion and secretions; cholestatic jaundice, rash (allergic) with and without pruritus, and mental depression.

A statistically significant association has been demonstrated between use of estrogen-progestin combination drugs and the serious adverse reactions of thrombophlebitis, pulmonary embolism and cerebral thrombosis and embolism. Therefore, patients on progestin therapy should be carefully observed.

Although available evidence is suggestive, a relationship has been neither confirmed nor refuted for the association of the serious adverse reaction of neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been observed in patients receiving estrogen-progestin combination drugs: rise in blood pressure in susceptible individuals; premenstrual-like syndrome, changes in libido; changes in appetite, cystitis-like syndrome, headache; nervousness; dizziness; fatigue; backache, hirsutism; loss of scalp hair; erythema multiforme; erythema nodosum; hemorrhagic eruption; and itching. Therefore, observe patients on progestin therapy carefully.

The following laboratory results may be altered by the use of estrogen-progestin combination drugs: increased sulfobromophthalein and other hepatic function tests; coagulation tests (increase in prothrombin factors VII, VIII, IX and X), metyrapone test; pregnanediol determination; thyroid function tests (increase in PBI, and butanol extractable protein bound iodine and decrease in T^3 uptake values).

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FETUS, PLACENTA, AND NEWBORN

Cardiac output in the fetal lamb: The effects of spontaneous and induced changes of heart rate on right and left ventricular output

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Right and left ventricular outputs were measured continuously in fetal lambs in utero for 2 to 21 days after implantation of electromagnetic flow transducers around the main pulmonary trunk and ascending aorta. Surface pacing electrodes were implanted on the left and right atria. The intact cervical vagus nerve was stimulated by electrodes implanted around the nerve. Spontaneous increases in fetal heart rate were associated with increases in both right and left ventricular outputs and spontaneous falls in heart rate with reduction in both outputs. There was a linear relationship between fetal heart rate and ventricular outputs. Vagal stimulation reduced fetal heart rate with a consistent and linear fall in ventricular output. Left atrial pacing increased right ventricular output by 15 to 20 per cent but produced a variable response, usually a fall, in left ventricular output. Right atrial pacing resulted in increases in both outputs but the increase in right ventricular output was less than during left atrial pacing at the same rate. The difference in response of ventricular outputs to induced as compared to spontaneous heart rate changes relates to the effects on left and right atrial phasic pressure relationships. These changes probably alter blood flow patterns across the foramen ovale.

MEASUREMENT of cardiac output in fetal animals has been hampered by difficulties in access to the fetus and by the presence of large central shunts in the fetal circulation. Most previous measurements of cardiac output have been made acutely in fetal lambs exteriorized from the uterus, with umbilical-placental flow maintained. The effects of anesthesia, removal of the fetus from the uterus, and surgical manipulation on cardiovascular function have not been assessed fully. It has been shown, however, that exteriorization of the fetus results in a progressive fall in umbilical blood flow.[1] The presence of large shunts through the foramen ovale and ductus arteriosus has precluded the application of methods used in adult animals. Modifications of the Fick[2] and indicator dilution techniques[3] have been used to measure cardiac output in exteriorized fetuses. Recently we described a method for measuring cardiac output in fetal lambs in which catheters had been chronically maintained in utero and

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radionuclide-labeled microspheres injected into a lower and upper limb or jugular vein.[4] The microsphere method, as well as the Fick and indicator dilution techniques, have the disadvantage that only single measurements can be made over limited periods of time, and rapid and sequential changes cannot be observed. Assali and associates[5] introduced the use of electromagnetic flowmeters to study ascending aortic and pulmonary trunk flow in fetal lambs. Although they could observe flows continuously, the combined outputs of the left and right ventricles were considerably lower than values reported for fetal lambs in utero.[4] Exteriorization of the fetus, the acute surgical procedure of thoracotomy, and extensive dissection of the great vessels probably influenced cardiovascular function profoundly.

In an attempt to study cardiovascular function in the fetus, in utero, in as undisturbed a state as possible, we have applied electromagnetic flow transducers of the cuff type around the main pulmonary trunk and the ascending aorta of fetal lambs and allowed them to recover from surgery. During continuous observations of right or left ventricular output we noted fluctuations associated with changes in heart rate. We have examined the effects of a spontaneous increase or decrease of heart rate on right and left ventricular output. We also induced changes in heart rate by electrically pacing the left or right atrium and stimulating the vagus nerve and studied the effects of these changes on right and left ventricular output.

Methods and materials

We studied the fetuses of 23 pregnant sheep of Dorset, Hampshire, or mixed strains. The gestational ages of the lambs were known to within 4 to 5 days of the date of conception, and ranged from 112 to 130 days (0.75 to 0.85 gestation).

Instrumentation of the fetus. The ewes were starved for periods varying from 24 to 48 hours prior to the performance of surgery. Strict aseptic precautions were observed throughout all surgical procedures. The mother was blindfolded and lower body analgesia accomplished by low spinal or, more recently, epidural anesthesia with 2 ml. (20 mg.) of tetracaine hydrochloride (Pontocaine). Polyvinyl catheters (I.D. 0.050″, O.D. 0.090″) were inserted into a maternal hindlimb artery and vein and advanced to the descending aorta and inferior vena cava. The ewe was then placed supine and through a midline abdominal incision the uterus was exposed. Sodium pentobarbital was given intravenously to the ewe in amounts of 200 to 300 mg. if she seemed restless or struggled. All fetal surgical procedures were performed with 0.5 per cent lidocaine hydrochloride for local anesthesia. A fetal hindlimb was located and exposed through a small (2 to 3 cm.) uterine incision. Polyvinyl catheters (I.D. 0.038″, O.D. 0.066″) were inserted into an artery and vein and advanced to the descending aorta and proximal inferior vena cava. The limb was returned to the uterus, a polyvinyl catheter (I.D. 0.050″, O.D. 0.090″) placed in the amniotic cavity, and the incision sutured.

A second 5 to 6 cm. incision was made in the uterine wall over the left side of the fetal chest, the left forelimb was extracted through this incision, and a thoracotomy was performed through the third or fourth intercostal space. The pericardium was incised over the pulmonary trunk from the region of the pulmonary valve to the ductus arteriosus, taking care to avoid damaging the vagus nerve. With minimal blunt dissection, the pulmonary trunk was separated from the underlying ascending aorta and aortic isthmus; short lengths of polyvinyl tubing (I.D. 0.030″, O.D. 0.048″) were passed around the vessels selected for instrumentation. A cuff-type electromagnetic flow transducer which had been precalibrated in vitro was placed around the ascending aorta distal to the origin of the coronary arteries to measure left ventricular output, but excluding coronary blood flow, in 10 animals. Transducers with inside diameters of 5 to 7 mm. were required to provide good electrode contact with minimal vessel constriction. In eight animals, transducers with an internal diameter of 8 to 11 mm. were placed around the pulmonary trunk midway between the pulmonary valve and the origin of the pulmonary arteries to measure right ventricular output. In five fetuses, flow transducers were placed around both vessels. In those animals with a transducer around the pulmonary trunk, a polyvinyl catheter (I.D. 0.030″, O.D. 0.048″) was inserted, either directly into the right ventricle or indirectly across the pulmonary valve from the pulmonary trunk, through purse-string sutures in the wall. A catheter (I.D. 0.030″, O.D. 0.048″) was also inserted through a purse-string suture into the left atrium in 14 fetuses. Electrodes for pacing the left or right atrium were prepared by soldering 1 mm. diameter bulbs of platinum to No. 30 Teflon-coated wire and gas sterilized. In all animals two electrodes were sutured on the inner aspect of the pericardium overlying the left atrium so that they lay in contact with the atrium about 1 cm. apart; in five animals an additional two electrodes were applied similarly to the right atrium. The pericardium was not sutured. A No. 8 F. polyvinyl tube with multiple side openings was placed in the left pleural space and the ribs brought together, with care to avoid angling or distorting the position of the transducers; in some

instances it was necessary to excise a short segment of rib. In some fetuses, polyvinyl catheters (I.D. 0.038″, O.D. 0.066″) were placed into the exposed axillary artery and vein; then the muscle and skin were sutured. The neck was exposed through either the same or a separate small uterine incision in some animals and the left vagus nerve and trachea dissected. Two electrodes consisting of 3 to 4 mm. lengths of platinum wire soldered to No. 30 Teflon-coated wire were applied around the vagus nerve about 1 cm. apart. A polyvinyl catheter (I.D. 0.038″, O.D. 0.066″) with multiple side holes was inserted through a purse-string suture into the trachea and advanced until the tip lay within the thorax. The skin and uterine incisions were sutured and all the catheters and electrodes led to the left flank of the ewe. The flow-transducer cables were passed through small incisions (1 cm.) in the abdominal wall, and the catheters and electrodes were led through a cannula inserted through the skin and into the peritoneal cavity. The catheters and electrodes were protected by a Teflon cloth pocket sewn to the skin on the flank. The catheters were filled with heparin solution and sealed with metal plugs. One million units of penicillin G and 500 mg. of Kanamycin were administered to the sheep on the day of surgery and each day thereafter. Half the total amount was injected into the amniotic cavity catheter and the remainder into the maternal vein catheter.

Recordings. The mother was allowed to recover from the surgery and measurements were made daily while the sheep stood quietly in a stall. Phasic and mean flows were measured with Statham SP 2202 electromagnetic flowmeters. The flows measured with the precalibrated transducers and this flowmeter were accurate to within 5 per cent. The electrical zero was checked repeatedly and showed only minor daily variations from zero flow recorded during the diastolic phase of the cardiac cycle. Pressures were measured continuously from the fetal arterial and amniotic cavity catheters in all animals and from the right ventricular, tracheal, left atrial, and inferior vena caval catheters in the animals in which they were inserted. All pressures were measured with $P_{23}Db$ (Statham) pressure transducers. At the onset of each observation period a fetal femoral arterial blood sample was obtained for measurement of Pco_2, Po_2, and pH with a radiometer blood-gas analyzer with appropriate electrodes. All pressure and flow recordings were made on a Beckman direct-writing recorder. Fetal heart rate was monitored continuously with a cardiotachometer triggered from either the fetal arterial pressure or the electromagnetic flow signal. In some studies beat-to-beat stroke volume

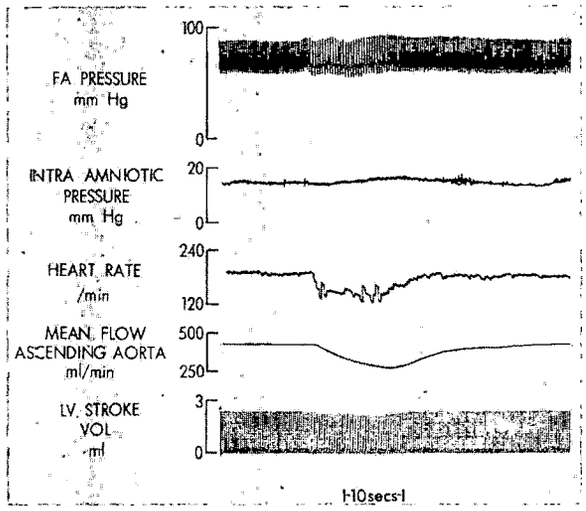


Fig. 1. Response of the fetal circulation to a 12 second long spontaneous fall in fetal heart rate; fetal femoral arterial *(FA)* and intra-amniotic pressures, heart rate, mean flow in the ascending aorta, and left ventricular *(LV)* stroke volume are shown.

was recorded by integrating the velocity signal from the flowmeter with a Beckman type 9873B integrator.

The pressures and flows were recorded continuously for varying periods of 15 minutes to 5 hours each day. Electrical pacing studies were started after permitting the animals to recover for at least 2 days after surgery. After obtaining control recordings for about 15 to 30 minutes, the left or right atrial electrode was stimulated at the resting heart rate and with frequencies increasing by 15 per minute to a maximum rate of 315 or 330 per minute. The stimuli were applied with a Grass 588 stimulator and an SIU5 stimulus isolation unit. The stimulation was applied at each rate until flows and pressures reached a new constant level; then it was stopped and pressures and flows allowed to return to control before the next stimulation at a higher rate was applied. In those animals with left and right atrial pacing electrodes, we sometimes alternated the pacing of each atrium at the same rate. On occasion the stimulation produced so much artifact in the flow signal that it was not possible to measure flow accurately; this was most often noted on the first few days after surgery and these observations were discarded. The stimuli consisted of 1 to 1.5 msec. impulses of 3 to 8 V. The voltage required to effect capture of the heart rate usually increased after a few days, but we were able to maintain effective pacing even 2 to 3 weeks after surgery. Vagal stimulation also was applied with the Grass stimulator and impulses of 3 msec. duration at 8 to 15 V. were applied at a frequency of 15 per second. The stimulation was maintained until a

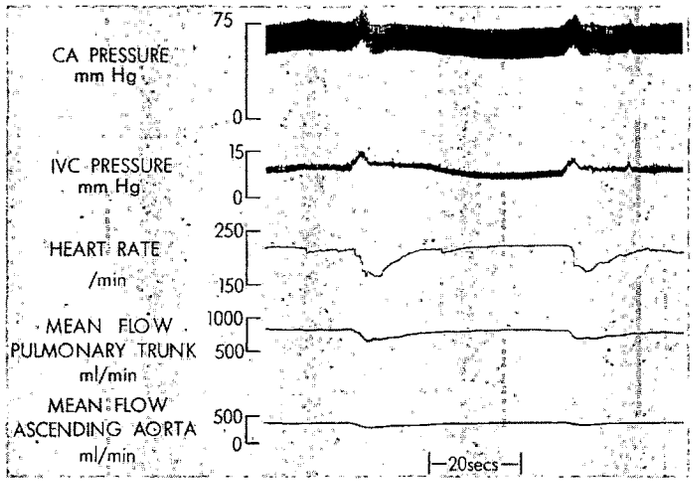


Fig. 2. Changes in the fetal circulation during a spontaneous fall in fetal heart rate; fetal carotid arterial *(CA)* and inferior vena caval *(IVC)* pressures, heart rate, and mean flow in both the main pulmonary trunk and ascending aorta are shown.

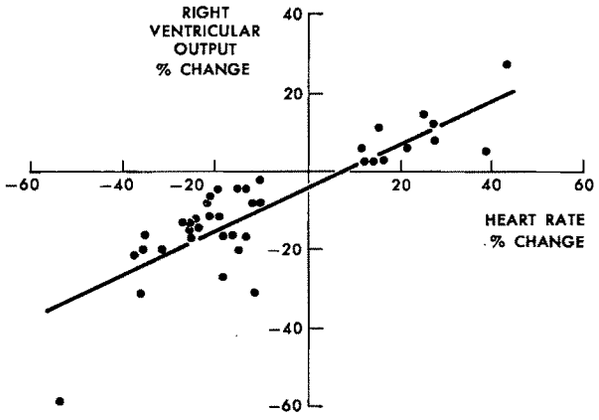


Fig. 3. The relationship between the percentage increases or percentage decreases (−) from resting of right ventricular output and heart rate during spontaneous changes in fetal heart rate is demonstrated.

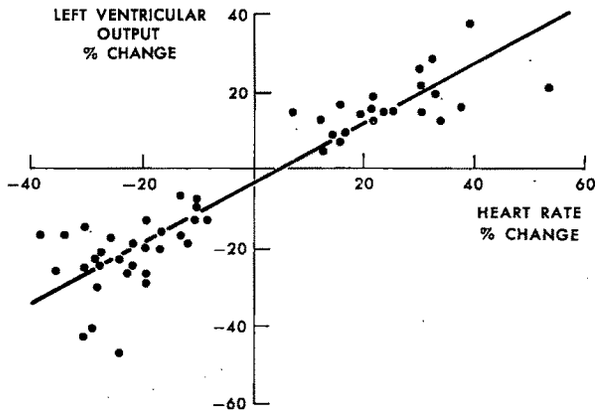


Fig. 4. The relationship between the percentage increases or percentage decreases (−) from resting of left ventricular output and heart rate during spontaneous changes in fetal heart rate is demonstrated.

steady level of pressures and flows was accomplished. During vagal stimulation it was noted that intrathoracic pressure as measured by the tracheal catheter increased in all animals; the magnitude of the increase varied in different fetuses from 2 to 3 mm. Hg to as much as 10 to 12 mm. Hg. In some fetuses a stable ventricular output was not achieved as intermittent expiratory movements with elevations of pressure of 10 to 20 mm. Hg occurred. In order to eliminate the effects of these respiratory changes on ventricular output, we administered intravenous succinylcholine chloride in doses of 2 mg. per kilogram directly to the fetus. This amount did not have any effect on resting heart rate, blood pressure, or ventricular outputs, nor did it alter the response to pacing the left or right atrium, or the circulatory changes associated with spontaneous variations of heart rate. The succinylcholine eliminated spontaneous respiratory movements and prevented the rise of intrathoracic pressure and the intermittent expiratory movements during vagal stimulation.

Results

Pressures, ventricular output, and heart rate were recorded on the first day after surgery and almost every day thereafter until fetal death or abortion occurred. Most intrauterine deaths were related to infection. All measurements reported were obtained when the fetuses had descending aortic $Po_2 > 20$ torr, $Pco_2 < 45$ torr, and $pH > 7.35$. There was no sig-

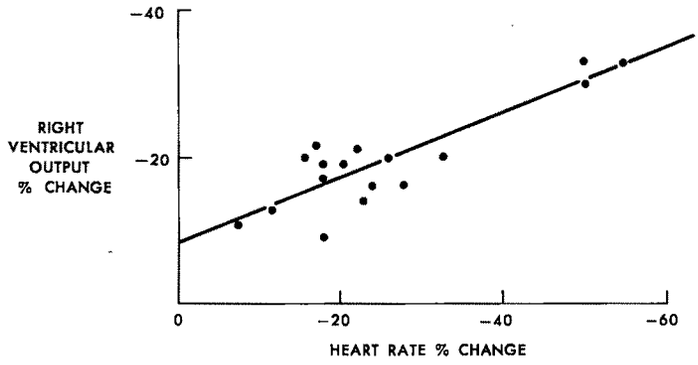


Fig. 5. The relationship between the percentage decreases from resting of right ventricular output and heart rate during electrical stimulation of the intact left vagus nerve is demonstrated.

nificant change in resting heart rate and aortic, left atrial, right ventricular, inferior vena caval, or amniotic cavity pressures after the first day of observation. In the six early studies, right ventricular output increased rapidly between the first and fifth days, but showed minor changes thereafter; in later studies, there was no significant change in flow after the first 2 days. There was also no difference in the spontaneous variation in flow associated with heart rate changes, nor in the responses to atrial pacing or vagal stimulation. We have therefore pooled the information obtained at various intervals after surgery. In analyzing the spontaneous variations in flow and heart rate, we measured the percentage increase or decrease from the resting level at the time of maximal change of heart rate. Similarly, during vagal stimulation and atrial pacing we measured the percentage change of left and right ventricular output and related it to the percentage change of heart rate.

Spontaneous changes in heart rate. Spontaneous increases in heart rate of greater than 10 per cent above resting level were invariably associated with an increase in both right and left ventricular outputs. The duration of the increased heart rate varied from 10 seconds to several minutes, and the increased output was maintained as long as the heart rate was elevated. In some instances the increased heart rate was associated with initiation of fetal respiratory movements, but episodic changes were observed even after respiratory movements were inhibited by muscular relaxation with succinylcholine. The increase in heart rate was often, but not always, associated with an elevation of aortic blood pressure. The rise of ventricular output consistently was related to the heart rate increase, whether or not fetal respiratory movements or a rise of blood pressure occurred.

Spontaneous decreases in heart rate of more than 10 per cent of resting level resulted in a fall in right and

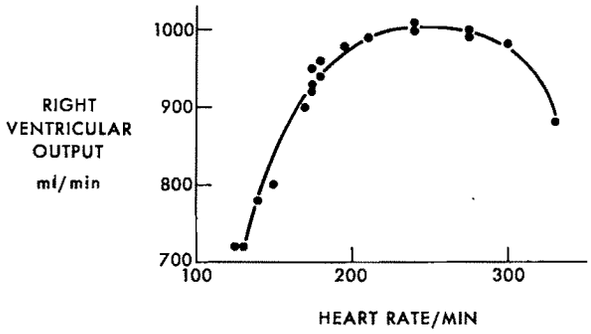


Fig. 6. The relationship between right ventricular output and heart rate in one fetus is shown. A marked rise in output occurs as the rate rises from 125 to 200 per minute. Above a rate of 300 per minute output falls.

left ventricular outputs. The reductions in heart rate were of 10 to 100 seconds duration and the flow was decreased during the whole period. These changes usually occurred independent of changes in amniotic cavity pressure, respiratory activity, or arterial pressure (Fig. 1). In several instances there was an associated increase in fetal arterial pressure and in intratracheal, left atrial, and inferior vena caval pressures in the first 3 to 5 seconds (Fig. 2). Administration of succinylcholine eliminated the respiratory and venous pressure changes but did not influence the flow response to reduction in heart rate.

There was a linear relationship between the percentage changes in heart rate and in right and left ventricular outputs. Fig. 3 shows the variation in right ventricular output (ordinate) associated with changes in heart rate (abscissa). The relationship is described by the equation $Y = 0.6X$ and the correlation coefficient is 0.83. Alteration of left ventricular output (ordinate) in relation to changes in heart rate (abscissa) is shown in Fig. 4. The relationship is described by the equation $Y = 0.74X$ and the correlation coefficient is 0.92.

The inferior vena caval and left atrial pressures were

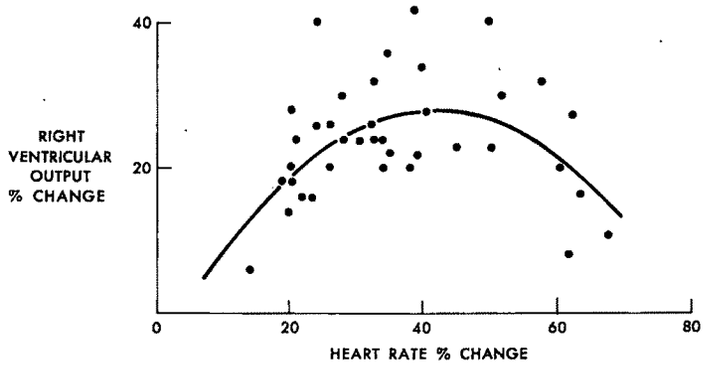


Fig. 7. The relationship between the percentage increases from resting of right ventricular output and heart rate during electrical pacing of the left atrium in 13 fetuses is demonstrated.

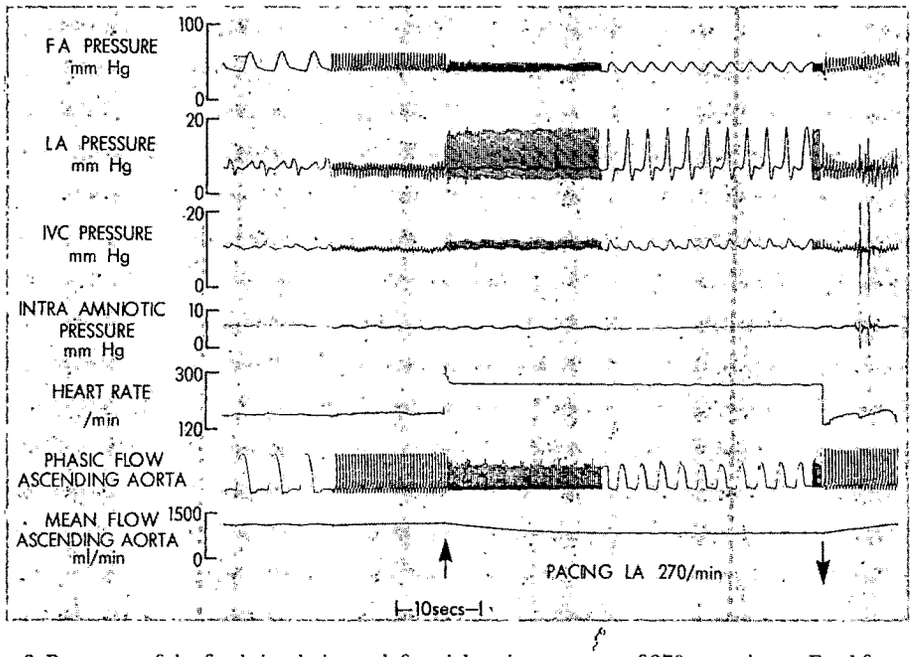


Fig. 8. Response of the fetal circulation to left atrial pacing at a rate of 270 per minute. Fetal femoral arterial *(FA)*, left atrial *(LA)*, inferior vena caval *(IVC)* and intra-amniotic pressures, heart rate, and phasic and mean flow in the ascending aorta are shown.

measured simultaneously in 14 fetuses. Pressure was measured in the proximal inferior vena cava in preference to the right atrium, since left atrial filling is derived largely from the inferior vena cava directly through the foramen ovale in the fetus. A mean pressure difference of 2 to 3 mm. Hg was recorded between the inferior vena cava and the left atrium (Fig. 12, *A*). There was no change in the mean pressure difference nor in the phasic pressure relationship between the vena cava and the left atrium during spontaneous changes in heart rate.

Cervical vagal stimulation. In these studies the intact vagus nerve was stimulated, so that cardiac slowing could have resulted directly from efferent fiber stimulation or through afferent fibers passing to cerebral vasomotor centers. Vagus nerve stimulation produced an elevation in arterial pressure which was not avoided by succinylcholine administration. Increases in tracheal and venous pressures associated with vagal stimulation were inhibited by succinylcholine. Vagal stimulation adequate to cause a reduction in heart rate produced a consistent fall in ventricular output. When vagal escape occurred the recovery of heart rate was associated with a parallel rise in flow. There was a linear relationship between the heart rate (abscissa) and right ventricular output (ordinate) as seen in Fig. 5. This is expressed by the equation $-Y = -0.4X - 8.5$, and the correlation coefficient is 0.86. The fact that the line intercepts with the flow axis on the negative side of zero suggests that

other factors, in addition to reduction of heart rate, influence right ventricular output during vagal stimulation. Vagal stimulation studies were conducted only in one animal in which left ventricular output was measured; similar changes were observed.

Left and right atrial pacing. The effects of left atrial pacing were studied in all the fetuses, but right atrial pacing studies were performed in only five animals to evaluate the differences in responses. Left atrial pacing resulted in a consistent increase in right ventricular output. In most of the studies, resting heart rate ranged between 160 and 180 per minute. Right ventricular output increased as rate was raised to 240 to 270 per minute, by an average of 15 to 20 per cent of the resting flow, maintained a plateau to rates of 300 to 315 per minute, and then fell with further increases of rate. A typical example of this response is shown in Fig. 6. The percentage increase in right ventricular output is related in a curvilinear manner (second-order regression) to the percentage increase in heart rate above resting levels, as shown in Fig. 7.

The effects of left atrial stimulation on left ventricular output were quite variable. In most studies, an increase in heart rate was associated with a progressive fall in left ventricular output. At rates of 270 to 330 per minute, left ventricular output usually fell by 50 to 60 per cent of resting levels (Fig. 8). In some instances, however, there was a small increase of left ventricular output of about 10 per cent at pacing rates of 210 to 240 per minute, but flow fell as rate was increased further. In view of the variable responses of left ventricular output to left atrial pacing at the same heart rate in different animals, no correlation could be defined. In the five animals in which flow transducers were placed on both the ascending aorta and the pulmonary trunk, the selective effects of left atrial pacing on left and right ventricular outputs were clearly demonstrated. Left ventricular output either fell, or increased minimally, while right ventricular output always increased (Fig. 9). If left ventricular output did increase slightly, right ventricular output increased much more.

We compared the effects of right and left atrial pacing in five fetuses. Right ventricular output consistently increased with both left and right atrial pacing, but the rise was always greater with left atrial pacing (Fig. 10). Similarly, left ventricular output rose slightly or fell during left atrial pacing, but increased during right atrial pacing. When the heart was paced at rates similar to resting rate, left atrial pacing usually produced a fall, but right atrial pacing caused a small rise, in left ventricular output (Fig. 11).

Fetal arterial pressure either increased slightly or did not change when pacing rate was slightly higher than resting rate (Fig. 11); with further increases, systolic pressure fell markedly, while diastolic pressure was maintained (Figs. 8 and 9).

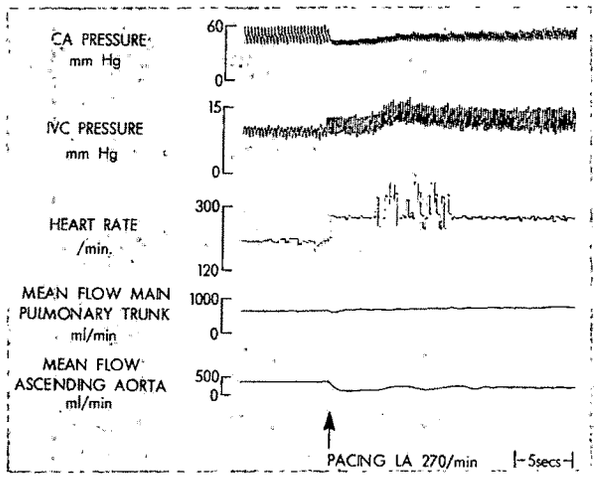


Fig. 9. Response of the fetal circulation to left atrial pacing at a rate of 270 per minute; fetal carotid arterial *(CA)* and inferior vena caval *(IVC)* pressures, heart rate, and mean flow in the main pulmonary trunk and ascending aorta are shown.

Left atrial and inferior vena caval pressures during atrial pacing. Inferior vena caval mean pressures were 2 to 3 mm. Hg higher than those in the left atrium in undisturbed fetuses. Left atrial pacing produced a reduction in the mean pressure difference to 0 to 1 mm. Hg and caused variable alterations in the phasic pressure contours. At lower pacing rates, small changes in left atrial and inferior vena caval pressures were observed (Fig. 11) but at high rates a very prominent a wave developed; this was more prominent in the left atrium than in the inferior vena cava (Figs. 8 and 9). The pressure contours differed during selective left and right atrial pacing in the same fetus (Fig. 10).

Rapid recordings of left atrial and inferior vena caval pressures were made using two carefully matched transducers with identical amplification. Inferior vena caval pressure exceeded that in the left atrium during all phases of the cardiac cycle in the resting fetus (Fig. 12, *A*). During left atrial pacing, however, left atrial pressure exceeded inferior vena caval pressure during some phase of the cardiac cycle, even though mean pressure usually was slightly higher in the inferior vena cava (Fig. 12, *B*). Right atrial pacing produced less striking changes in phasic pressure contours than left atrial pacing (Fig. 12, *C*).

Comment

The development of a model for measuring right and left ventricular output by chronic implantation of

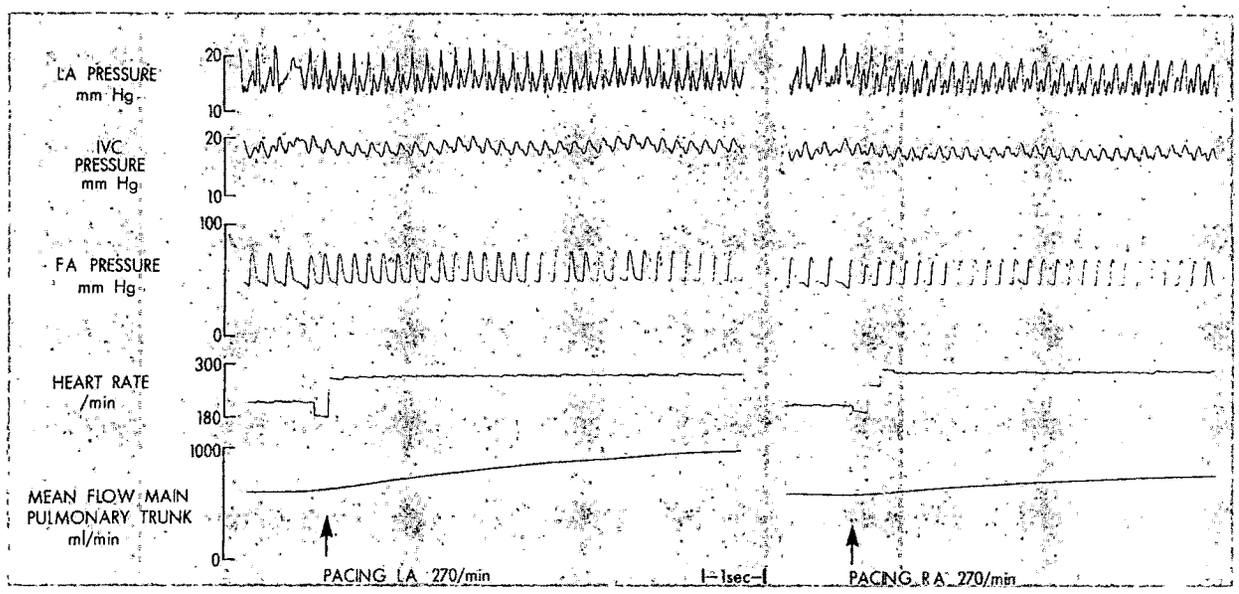


Fig. 10. Response of the fetal circulation, in the same fetus, to left atrial *(LA)* and right atrial *(RA)* pacing at a rate of 270 per minute; fetal left atrial *(LA)*, inferior vena caval *(IVC)*, and femoral arterial *(FA)* pressures, heart rate, and mean flow in the main pulmonary trunk are shown.

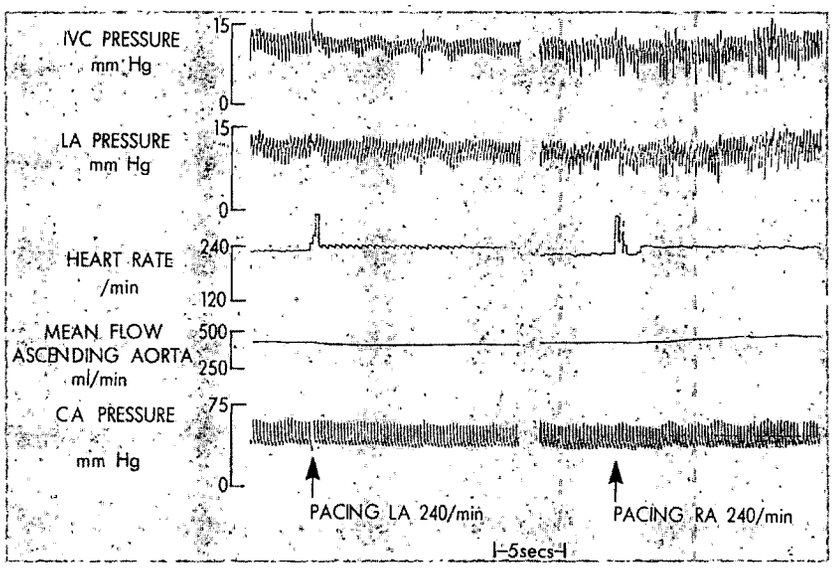


Fig. 11. Response of the fetal circulation in the same fetus to left atrial *(LA)* and right atrial *(RA)* pacing at a rate of 240 per minute; fetal inferior vena caval *(IVC)*, left atrial *(LA)*, and carotid arterial *(CA)* pressures, heart rate, and mean flow in the ascending aorta are shown.

electromagnetic flow transducers, has afforded the opportunity to examine instantaneous hemodynamic responses in the relatively undisturbed lamb in utero. The importance of permitting an adequate period of recovery after surgery is underscored by our observation that cardiac output is reduced during surgery and for several hours thereafter. Although right ventricular output was reduced for 2 to 5 days in our earlier preparations,[6] with improvement of technique, both right and left ventricular output recovered within 24 to 48 hours. This is supported by the finding that the measurements of cardiac output are similar to those obtained by the radionuclide-labeled microsphere technique in fetal lambs 2 to 3 weeks after minor surgery to place catheters in peripheral vessels.[7, 8] Other physiologic functions are disturbed in the fetus for several days after surgery; nitrogen metabolism may be altered[9] and corticosteroid secretion

increased.[10] It is possible that there may be some circulatory changes beyond the first day after surgery, but the fact that all the responses we have examined have been similar in the first few days to those observed 2 to 3 weeks after surgery, has suggested that the cardiovascular system is relatively normal.

We do not know why spontaneous changes in fetal heart rate occur. Some episodes of tachycardia are associated with the onset of fetal respiratory movements and are probably related to changes in electrocortical activity.[11] However, many episodes are not associated with fetal respiratory activity, and tachycardia may not occur with respiratory movements. The frequent association of hypertension suggests that the episodes may be due to hypothalamic discharge, since the cardiovascular manifestations are similar to those observed with stimulation of electrodes placed in the hypothalamus of fetal lambs.[12]

Spontaneous bradycardia was not related to uterine activity in our studies, as no changes in amniotic cavity pressure were observed. Some of the episodes of slowing were associated with increased intratracheal pressures. It is possible that fetal grunting raised intrapleural pressure, resulting in decreased venous return, reduced cardiac output, and bradycardia, as occurs postnatally with the Valsalva maneuver. Blocking the respiratory pressure changes by muscular paralysis with succinylcholine did not, however, prevent the episodes of slowing. The changes could be associated with episodic vagal discharge, initiated centrally or reflexly.

Whatever the cause of the spontaneous variations in heart rate, right and left ventricular output invariably showed similar directional changes. These observations have important clinical implications, since it is possible to assess the magnitude of changes in cardiac output from the alterations of heart rate from resting levels. A reduction in cardiac output will result in a fall in umbilical blood flow with impaired placental gas exchange, or a decrease in fetal body flow with inadequate oxygen supply to the tissues.[13] Thus assessment of fetal heart rate and its changes provides a convenient measure of cardiovascular adequacy.

The effects of vagal stimulation on cardiac output are of considerable interest, since parasympathetic influence on the heart precedes sympathetic nervous activity.[14] The bradycardia associated with hypoxia largely is due to a baroreflex response to hypertension induced by peripheral vasoconstriction.[13] Although the reduction of cardiac output associated with vagal stimulation is produced mainly by the decrease in heart rate, our studies suggest that other mechanisms may also be involved. It is possible that vagal stimulation has

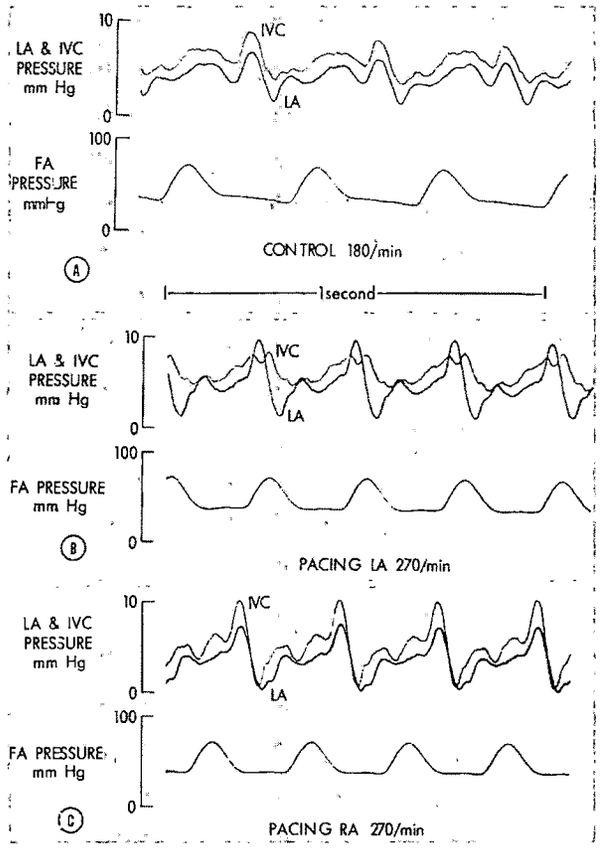


Fig. 12. Fetal left atrial *(LA)*, inferior vena caval *(IVC)*, and femoral arterial pressures are shown: *(A)* during a control period with a heart rate of 180 per minite; *(B)* during left atrial *(LA)* pacing at 270 per minute; and *(C)* during right atrial pacing at 270 per minute.

a direct depressant action on the myocardium, but the finding could be explained by parasympathetic-induced peripheral vasoconstriction. The increased afterload on the ventricles resulting from an increase in vascular resistance could reduce stroke volume.

The marked reduction in cardiac output associated with bradycardia in the fetus implies that the fetal heart has a limited ability to increase stroke volume. This is in striking contrast to the adult animal, in which stroke volume adjusts to changes in heart rate over a wide range thus maintaining a relatively constant cardiac output.[15] The restriction in stroke volume response to cardiac slowing could be due to immaturity of fetal myocardial development and lack of adequate sympathetic innervation to the ventricles.[6]

The effects of electrical pacing of the atria in the fetus were at variance with observations in adult animals. Douglas and associates[16] found no difference in the effects of left or right atrial pacing in adult dogs, even in the presence of small to moderate-sized atrial septal defects. We found consistently that right ven-

tricular output increased during pacing, but the rise was greater with left than with right atrial stimulation. Left ventricular output increased during right atrial pacing, but fell, or increased minimally, during left atrial pacing. The limitation of ventricular output response when the atrium which provides its inflow is paced, could be explained by an interference with effective filling. Since, in the fetus, both atria have a dominant a wave atrial systole may be very important in filling the ventricles in end-diastole. The relationship of the atrial contraction to ventricular pressure may be altered so that it does not provide as effective presystolic filling, and thus the volume of blood ejected by the ventricle will be reduced. Although this mechanism could explain the differences in the responses of the output of the right and left ventricles, it is more likely that they are related to variations in phasic pressure relationships between the left atrium and the inferior vena cava and right atrium. We have shown that the inferior vena caval pressure is normally higher than that in the left atrium throughout all phases of the cardiac cycle in the fetus; blood therefore flows through the foramen ovale into the left atrium. With left atrial pacing, the left atrial pressure may exceed that in the inferior vena cava during some periods, thus tending to close the foramen ovale, and interfere with left atrial filling and left ventricular output. The diversion of blood to the right atrium and through the tricuspid valve would favor right ventricular output. We favor this hypothesis, since we have shown that when left atrial pressure is raised in the fetus by increasing pulmonary blood flow by means of acetycholine infusion, there is a considerable reduction in the passage of inferior vena caval blood across the foramen ovale.[17]

These observations of the effects of atrial pacing on ventricular output in the fetus are important because they point out that atrial arrhythmias could have profound influences on the intracardiac distribution of flow in the fetus. Furthermore, they underscore the fact that physiologic responses in the adult are not necessarily applicable to the fetal circulation.

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Development of a special electrode for continuous subcutaneous pH measurement in the infant scalp

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Using a combined special glass electrode it is possible to monitor pH ratios and pH variation in the subcutaneous tissue of the infant scalp continuously. Tests on a normal sample of newborn babies immediately after birth showed a significant correlation between tissue pH and capillary blood pH, with the trend of pH variation being broadly similar in both measurement media.

FETAL-SCALP blood sampling[1, 2] has been widely adopted as a means of monitoring the condition of the fetus during labor by intermittent analysis of acid-base components. The usefulness of this method is limited by the fact that no information is available during the interval between two blood samples. It therefore seemed to us desirable to replace the intermittent method by a continuous, quickly readable, and labor-saving method of recording pH.

Material and methods

On the hypothetical assumption that pH ratios in subcutaneous tissue could also provide clinically useful pointers to the child's metabolic situation, a special glass electrode was constructed (Fig. 1), the point of which is inserted into the subcutaneous tissue of the child's scalp and fixed with spiral springs.

Measurement of pH is based on the electrometric principle. The menstruation chain consists of a combined glass electrode with indicator and direct recorder. The chief constructional novelty of our electrode lies in the combination of both electrodes in a spherical mensuration head. This construction, i.e., reduction of the current key to a minimal distance of 2 to 3 mm., makes it possible to eliminate interference potential from muscle-action currents.

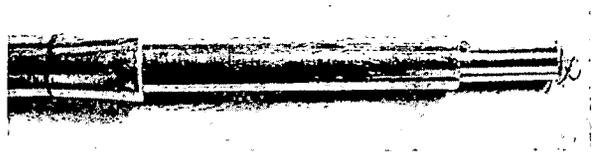


Fig. 1. Special glass electrode for continuous pH measurement in the subcutaneous tissue of the infant scalp.

The accuracy of measurement of the pH electrode described varies during continuous recording over a 2 hour period in standard buffer solution by 0.007 ± 0.002 pH units ($\bar{x}$ ± S.D.).

The investigation under discussion examined the practical applicability of the special probe in the subcutaneous tissue of the scalps of newborn babies. In a sample of 10 children born normally and with an Apgar score of 9/10//10 the electrode point was inserted in the scalp to a depth of approximately 3 mm. immediately after birth and subcutaneous pH recorded continuously over a period of 60 minutes. To clarify the correlation between subcutaneous pH and capillary blood pH, every 5 minutes a sample of heel blood was taken for pH analysis as well.

Results

As can be seen from Figs. 2 and 3, the pH mensuration chain gives relatively interference-free recording curves for pH ratios in subcutaneous tissue. The curve trend in the postpartum phase is similar in all 10 cases and is characterized by a progressive pH increase from

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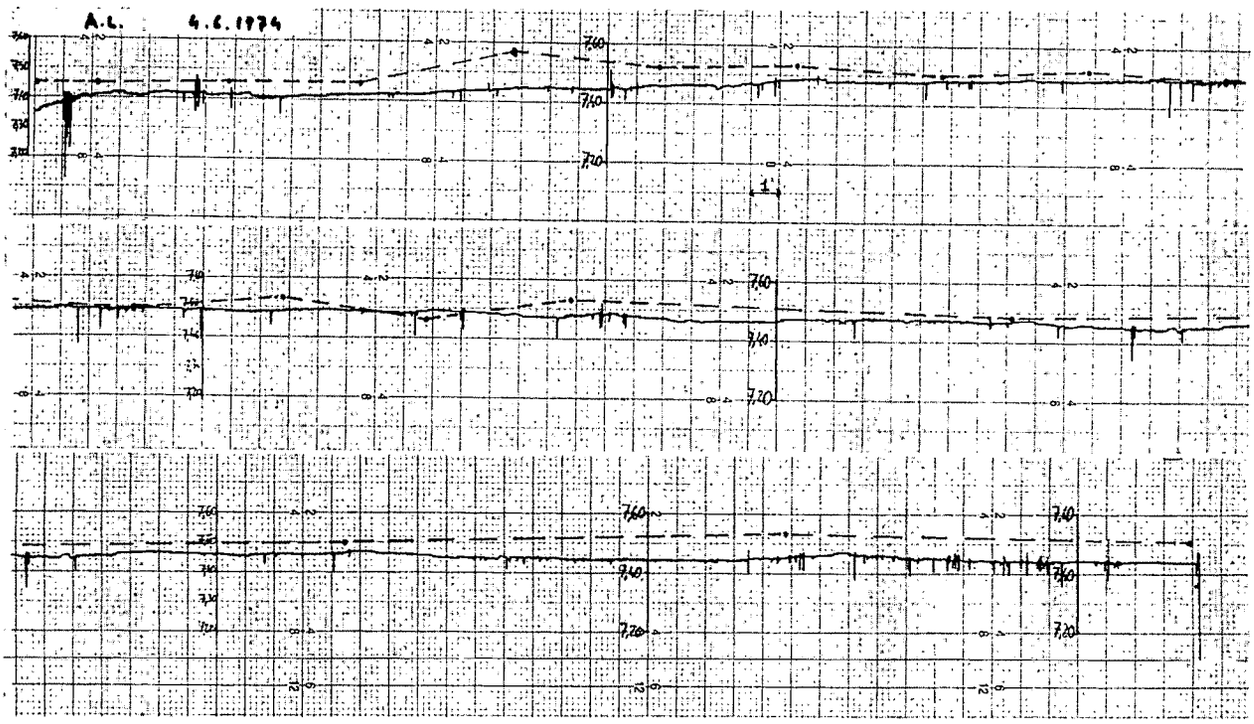


Fig. 2. Child A. L.: Sections of a continuous subcutaneous pH measurement reading immediately after birth. Dashed line shows corresponding capillary blood pH values.

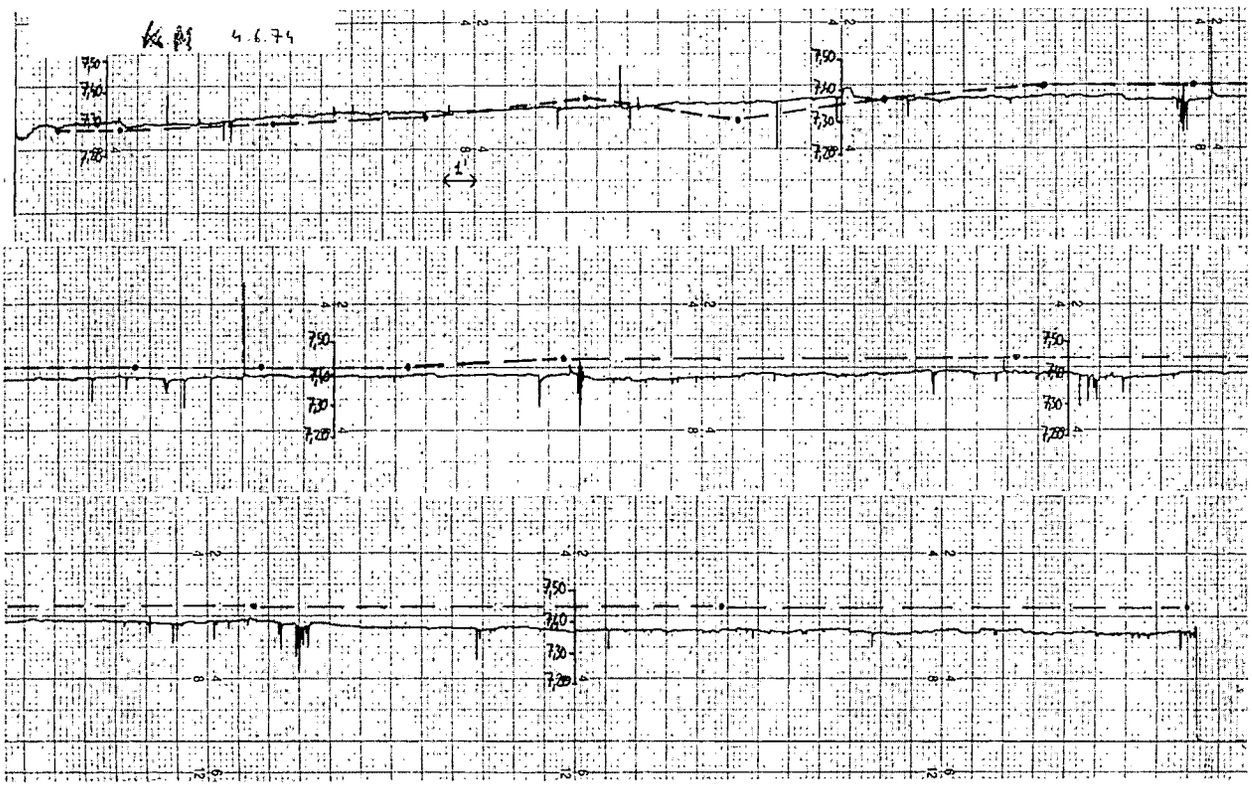


Fig. 3. Child K. M.: Continuous subcutaneous pH measurement over a 2 hour period. Dashed line shows corresponding capillary blood pH values.

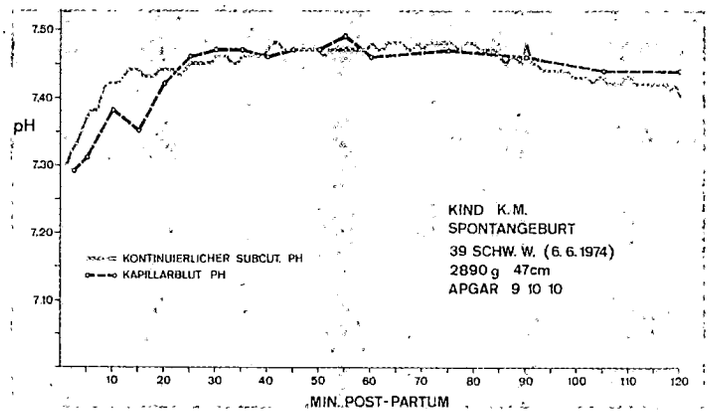


Fig. 4. Child K. M. (same case as in Fig. 3): Graph showing pH trend in subcutaneous tissue and in capillary blood, recorded over a 2 hour period (breakdown of subcutaneous recording curve into 1 minute values.

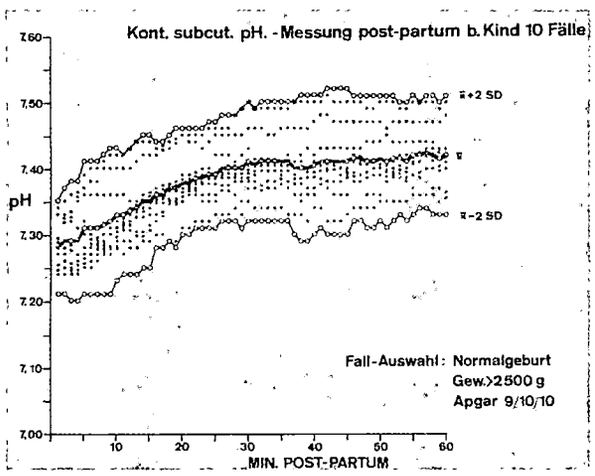


Fig. 5. Subcutaneous pH mean values with double standard deviation in a normal sample (10 children with Apgar score 9/10/10).

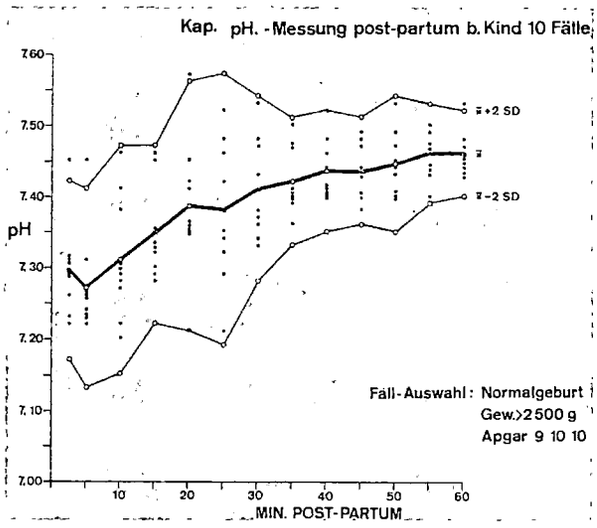


Fig. 6. Capillary blood pH mean values with double standard deviation for the same normal sample as in Fig. 5 (10 children with Apgar score 9/10/10).

$\bar{x}_1$ 7.29 ± 0.04 (immediately post partum) to $\bar{x}_2$ 7.42 ± 0.05 pH (60 minutes post partum).

As has been mentioned already, in each case capillary blood pH was controlled also, at intervals of 5 minutes. The close correlation of pH ratios in both measurement media can be seen in Fig. 4. Basically pH values for the normal sample examined lie in the same pH range, which means that acidosis adjustment in subcutaneous tissue can occur with a delay of approximately 2 to 5 minutes.

For the 10 normal cases mean values with standard deviation were calculated for subcutaneous and for capillary pH. In addition the recording curves were broken down into 1 minute values. The results are shown in Figs. 5 and 6. Mean values in the two measurement media differed by a maximum of 0.04 pH units. The curves follow the same time pattern. The 95 per cent deviation range is less wide in the case of continuous subcutaneous pH recording than in the case of intermittent monitoring of capillary blood pH.

Correlation analysis between subcutaneous pH mean value and capillary blood pH mean value produced a correlation coefficient of $r = 0.965$ ($p < 0.001$).

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Maternal endotoxemia, fetal anomalies, and central nervous system damage: A rat model of a human problem

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Endotoxemia is a common consequence of the gram-negative urinary tract infections that complicate human pregnancies. Only rarely, however, have the effects of maternal endotoxemia been evaluated by animal experiments or by human investigations. Data of the Collaborative Perinatal Study suggest an association between maternal endotoxemia and fetal central nervous system damage. For these reasons we performed controlled studies of the fetal effects of treatment of pregnant rats, at appropriate gestational ages, with E. coli *endotoxin. We found a maximum 7 per cent incidence of fetal anomalies in the treated animals but no anomalies in controls. Placental light microscopy examinations indicated the mechanism to include Shwartzman-like phenomena. It has been shown by others that neonatal endotoxemia produces periventricular leukomalacia. We obtained an incidence of neuronal necrosis in treated fetuses that was 10 times greater than in control fetuses. It is therefore of importance that additional studies of the pathologic effects of endotoxin be performed.*

IN THE Collaborative Perinatal Study of the National Institute of Neurological Diseases and Stroke, fever with positive urine culture is stated to occur in 1.27 per cent of pregnant white women and in 1.74 per cent of pregnant black women.[14] This complication of pregnancy suggests an occurrence of maternal endotoxemia resulting from the gram-negative organisms that typically cause urinary tract infections. Since pregnant women with fever and bacteriuria give birth to a higher incidence of infants with neurologic defects at 1 year of age than do mothers free of urinary tract infection,[14] it appears that human maternal endotoxemia is associated with fetal central nervous system damage. Endotoxin has been shown to induce placental damage and abortion in experimental animals.[7, 13, 20, 23] Moreover, even a single injection of the active lipopolysaccharide fraction can cause the generalized Shwartzman reaction in pregnant animals.[13, 21]

Kass[8] has reported bacteriuria in 6 to 7 per cent of pregnant women, many of whom delivered premature infants. Following that investigation contradictory results were obtained by other investigators. Kincaid-Smith and Bullen[9] observed a higher percentage of prematurity associated with maternal bacteriuria but Wilson and associates[25] did not.

In a study of chronic bacteriuria of pregnancy Patrick[18] did not find an increased incidence of prematurity or perinatal death but found an increased malformation rate of 5.4 per cent as opposed to 0.8 per cent in her control group. Most of the anomalies were of the central nervous system. Ohba[16] also observed central nervous system anomalies in fetal mice whose mothers had been treated with *E. coli* endotoxin. Other than for these studies there is a lack of critical investigation both in human beings and animals of the association of congenital anomalies with maternal bacteriuria and endotoxemia. We decided to design a study to investigate whether results of an animal experiment support the apparent association between human fetal damage and maternal endotoxemia. We chose the rat arbitrarily and, although aware that strain

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Table I. Fetal and placental weight on day 21 of pregnancy, litter average, resorption rate, and rate of anomalies in control and experimental animals

Treatment	*Average fetal wt. (Gm.)*	*Average placental wt. (mg.)*	*Litter average (living fetuses)*	*Rate of resorption*	*Rate of fetal anomalies*
Group I: 0.15 mg. *E. coli* endotoxin on days 14, 17 (6)*	4.39 ± 0.20† (51)‡	585 ± 33	9.3	11/67 16.4%	0/56 0%
Group II: 0.15 mg. *E. coli* endotoxin on days 10, 13, 16 or 11, 14, 17 (9)*	4.24 ± 0.46 (70)‡	497 ± 50	8.2	19/93 20.4%	5/74 6.8%
Group III: 0.15 mg. *E. coli* endotoxin on days 8, 10, 12 (6)*	4.12 ± 0.27 (71)‡	622 ± 25	11.8	4/75 5.3%	5/71 7.0%
Controls (Group IV): 0.15 ml. normal saline ×3 or no treatment (11)*	4.86 ± 0.40 (53)‡	495 ± 56	9.6	2/108 1.9%	0/106 0%

*Numbers in parentheses represent the mother rats with living fetuses.
†S.E. calculated by Mantel's method (1951).
‡Numbers in parentheses represent the number of fetuses killed on day 21.

Table II. Group correlation between rate of placental or yolk sac damage vs. neuronal necrosis

Treatment	*Moderate or severe placental damage*	*Moderate or severe yolk sac placenta damage*	*Moderate or severe neuronal necrosis*
Group I: 0.15 mg. *E. coli* endotoxin, days 14 and 17	27/27	14/27 51.9%	2+1†/30 10.0%
Group II: 0.15 mg. *E. coli* endotoxin, days 10, 13, 16 or 11, 14, 17	(41 + 4*)/45	26/41 63.4%	26+2†/39 71.8%
Group III: 0.15 mg. *E. coli* endotoxin, days 8, 10, 12	(30 + 10*)/40	13/40 28.3%	17/33 51.5%
Controls (Group IV): 0.15 ml. normal saline × 3 or no treatment	4*/38	3*/38 7.8%	4/54 7.4%

*Indicates slight damage.
†Severe neuronal necrosis in spinal cord only.

differences in results might occur, were not concerned specifically to investigate that possibility.

Materials and methods

White rats of the Sprague-Dawley and Wistar strains were used for the experiment. The animals were mated and the first day of pregnancy assessed by the finding of sperm in vaginal smears. A 0.5 mg. dose of endotoxin (*E. coli* endotoxin, 026-B6, Difco Laboratories, Detroit, Michigan) was initially administered intraperitoneally (i.p.) to the pregnant rats. This dose was too high, being lethal occasionally even after only one dose. A 0.25 mg. dose, injected on day 13 or 14 of gestation, did not kill the animals, but many aborted. We therefore further reduced the dose to 0.15 mg.

Rats were divided into the following groups: Group I, eight pregnant rats injected with 0.15 mg. of endotoxin i.p. on days 14 and 17 of gestation; Group II, 15 pregnant rats receiving the same dose i.p. on days 11, 14, and 17 of gestation (eight rats) or gestational days 10, 13, and 16 (seven rats); Group III, seven pregnant rats injected with 0.15 mg. of endotoxin i.p. on days 8, 10 and 12 of gestation; Group IV, 12 control rats which either were not treated (six rats) or which were given normal saline (six rats) i.p. on gestational days 11, 14, and 17.

All but five rats were killed on gestational day 21, following ether anesthesia. The abdomen was opened and resorption sites and living fetuses were counted. The placentas and attached uterine walls were examined by light microscopy as were the maternal kidneys, adrenal glands, lungs, livers, and spleens. The methods for the light microscopy examination included fixation in Bouin's solution and standard procedures of processing the tissue into paraffin

Table III. The effects of 0.15 mg. of *E. coli* endotoxin injected on days 14 and 17 of gestation on placental structure and fetal outcome

No. of living fetuses in the litter	*Pathology of labyrinthine placenta*	*Decidual lesions*	*Balloon degeneration of yolk sac epithelium*	*Lesion of maternal organs*	*Fetal lesion*
5 (1)*	Slight focal intervillous leukostasis; moderate glycogen cell necrosis	Severe acute necrosis with associated fibrin thrombi	None	None	No anomalies
7 (3)	Moderately severe necrosis of glycogen cells and quasisyncytium	Moderate acute necrosis	None	None	No anomalies
9 (2)	Severe necrosis of glycogen cells and quasisyncytium	Moderate acute necrosis	Moderate with associated leukocytic infiltration beneath epithelium	Slight fibrin in 1 renal vein	No anomalies
12† (3)	—	—	—	None	No anomalies; 1 fetus with intra-abdominal hemorrhages
16 (2)	Severe placental ischemia moderate necrosis of glycogen cells and quasisyncytium	Severe acute necrosis with associated fibrin thrombi	Slight to moderate, focal	None	No anomalies

*Numbers in parentheses represent the resorption in each litter.

†For technical reasons these placentas are not included.

Table IV. The effects of 0.15 mg. of *E. coli* endotoxin injected on days 10, 13, and 16 or 11, 14, and 17 of gestation on placental structure and fetal outcome

No. of living fetuses in the litter	*Pathology of labyrinthine placenta*	*Decidual lesions*	*Balloon degeneration of yolk sac epithelium*	*Lesions of maternal organs*	*Fetal lesions*
4 (0*)	Severe glycogen cell necrosis, moderate focal leukostasis, few fibrin thrombi	Severe acute necrosis with fibrin thrombi	Moderate	None	1 fetus with hydrocephalus
5 (1)	Severe necrosis of glycogen cells and quasisyncytium; moderate placental ischemia and leukostatis	Severe acute necrosis with few fibrin thrombi	Severe with associated leukocytic infiltration beneath epithelium	Slight fibrin in 1 renal vein	1 fetus with hydrocephalus; 1 fetus with microcephaly, brain dysplasia, absent rt. urogenital ridge, and rt. anophthalmia
9 (1)	Moderate necrosis of glycogen cells and quasisyncytium	Moderate acute necrosis	Moderate	None	No anomalies; 1 fetus with extensive intra-abdominal hemorrhage
6 (4)	Severe necrosis of glycogen cells and quasisyncytium; focal placental ischemia	Moderately severe acute necrosis	Moderate	None	No anomalies; 1 fetus with extensive subcutaneous hemorrhage
7 (4)	Severe necrosis of glycogen cells and quasisyncytium; focal placental ischemia	Severe acute necrosis	Severe with leukostasis	None	None
13 (1)	Moderate necrosis of glycogen cells and quasisyncytium	Moderate acute necrosis	Slight	Reactive spleen	None
10 (1)	Moderate necrosis of glycogen cells	Severe acute necrosis	None	None	None
14 (0)	Moderate necrosis of glycogen cells and quasisyncytium	Moderate acute necrosis	Moderate	None	1 fetus with hydrocephaly; 1 fetus with microcephaly
2 (7)	Moderate necrosis of glycogen cells and quasisyncytium	Moderate acute necrosis	None	None	None

*Numbers in parentheses represent the resorptions in each litter.

Table V. The effects of 0.15 mg. of *E. coli* endotoxin injected on days 8, 10 and 12 of gestation on placental structure and fetal outcome

No. of living fetuses in litter	*Pathology of labyrinthine placenta*	*Decidual lesions*	*Balloon degeneration of yolk sac epithelium*	*Lesions of maternal organs*	*Fetal lesions*
13 (0*)	Moderate necrosis of glycogen cells and quasisyncytium	Moderate acute necrosis	Moderate, focal	None	1 fetus with hydrocephalus and rt. hydronephrosis; 1 fetus with single left lateral ventricle of brain; 1 fetus with hydrocephalus
16 (0)	Slight glycogen cell necrosis	Slight acute necrosis	None	None	None
11 (0)	Severe glycogen cell necrosis	Moderate acute necrosis with fibrin thrombi	Slight or moderate	None	1 fetus with hydrocephalus; 1 fetus with hemorrhage in rt. forearm, pericardium, mediastinum, and lungs
12 (1)	Severe chorioamnionitis; moderate glycogen cell necrosis	Severe deciduitis with acute necrosis	Moderate focal	Focal pneumonia	No anomalies; 1 fetus with hemorrhage in pericardium and lungs
7 (2)	Moderate necrosis of glycogen cells and quasisyncytium; moderate placental ischemia, focal	Decidual microabscesses, severe acute decidual necrosis	Slight, focal	Reactive spleen	1 fetus: lt. microphthalmia and small auricle
12 (1)	Moderately severe necrosis of glycogen cells and quasisyncytium; leukostasis in labyrinth and focal placental ischemia	Severe acute necrosis	Moderate diffuse and focal	None	None

*Numbers in parentheses represent the resorptions in each litter.

blocks, 6μ sectioning, and staining with hematoxylin and eosin. Following examination of the fetuses by the sectioning method described by Wilson,[24] transverse sections of the brains through the lateral ventricles at the level of the trigeminal ganglion and sections of the thoracic spinal cord were evaluated for neuronal necrosis. On no occasion did at least one of the examining investigators have foreknowledge as to whether the sections to be evaluated were of the experimental or of the control animals. The finding of three to four necrotic neurons in one section of the diencephalon or one to two in the spinal cord was considered as moderate neuronal necrosis, and the presence of five or more necrotic neurons in the diencephalon or three or more in the spinal cord was considered as severe neuronal necrosis.

Results

Maternal toxicity. Two pregnant rats died during the course of the experiment, one control and one from experimental Group I. Nine rats completely resorbed their litters or aborted: one from Group I, one from Group III, and seven from Group II. The placentas of the resorbed fetuses showed widespread severe ischemic necrosis, occasionally associated with fibrin thrombi. The experimental rats which did not resorb all their fetuses gained more than 100 Gm. of weight during pregnancy, as did the controls. However, two to three hours after each endotoxin injection the animals were lethargic, weak, and uninterested in their environment. These effects lasted less than 24 hours.

Fetuses and placentas. The mean fetal and placental weights on the beginning of day 21 of pregnancy were similar in the experimental and control animals (Table I). The litter average in Groups I and II was lower than in the controls and the resorption rates, excluding the animals that resorbed all their litter, were 16.4 and 20.4 per cent, respectively (Table I). The resorption rate in experimental Group III rats was only 5.3 per cent (Table I).

The placentas of all experimental animals showed severe pathologic changes: decidual necrosis, necrosis of glycogen cells (clear cells) and quasisyncytium (junctional zone damage), congestion of the labyrinth with occasional ischemic areas, and balloon degeneration of epithelial cells of the yolk sac placentas (Tables II to V). In the controls, slight placental damage was

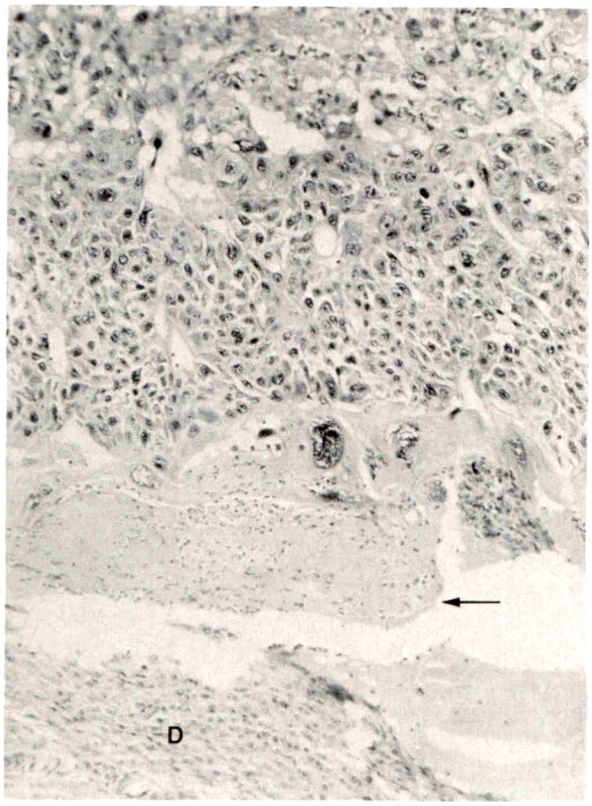


Fig. 1. Placental floor. Note the fibrin thrombus in a maternal vessel (arrow) overlying the decidua *(D)*. (Hematoxylin and eosin; original magnification ×160.)

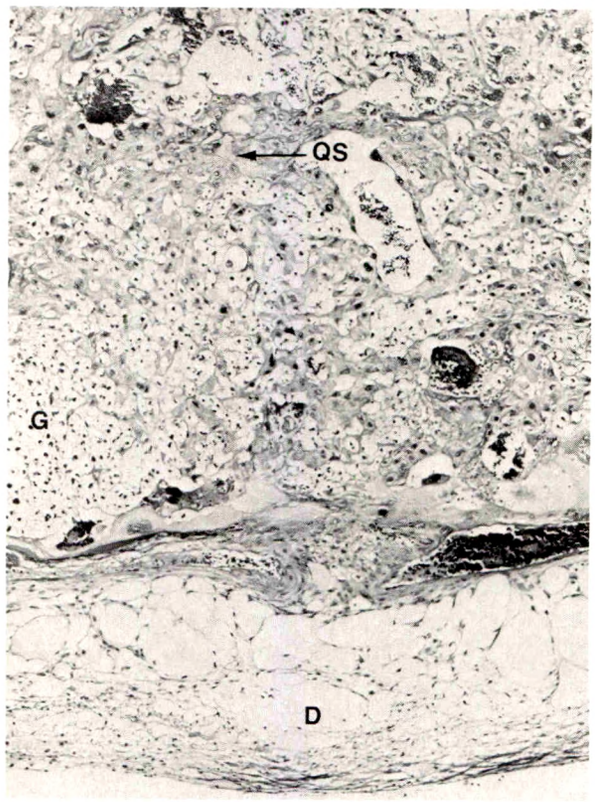


Fig. 2. Placenta floor, junctional zone, and labyrinth. Note the severe acute infarction of the decidua *(D)*, the glycogen cells *(G)*, and the quasisyncytium *(QS)*. (Hematoxylin and eosin; original magnification × 70.)

present in two animals (Table II). Maternal organs of control rats were normal except in one rat in which extramedullary hematopoiesis in liver and spleen, bronchitis, and bronchiolitis were present.

In the animals from the first experimental group (Table III), the placentas had severe lesions: moderate or severe maternal floor infarction occasionally associated with fibrin thrombi, severe necrosis of glycogen cells, ischemia, and congestion of the labyrinth (Figs. 1 and 2). In two rats of experimental Group I, yolk sac lesions were present—ballooning of the yolk sac epithelial cells (Figs. 3 and 4) with leukocytic infiltration beneath the epithelium (Table III).

The most severe placental lesions were found in the experimental Group II animals (Table IV). Fibrin thrombi in decidual vessels were more frequently observed, and in some animals occasional fibrin thrombi were additionally found in the labyrinth. The yolk sac placental damage, although similar to that of experimental Group I, was more severe. Placental damage (including yolk sac lesions) was prominent even in experimental Group III. In this last group one mother had microscopical evidence of pneumonia (Table V).

No disseminated fibrin thrombi were found in the organs examined from the rats of all three experimental groups. In only two rats were rare, early fibrin thrombi present. These were located in renal veins.

No gross anomalies were found in the 106 control fetuses. None of the 56 Group I fetuses was malformed (Tables I and III). Five out of 74 Group II fetuses were malformed (Tables I and IV): three had hydrocephaly (Fig. 5), one had microcephaly, and one had multiple anomalies—abnormal small head, severe brain dysplasia (Figs. 6 and 7), microphthalmia, and anophthalmia and agenesis of the left urogenital ridge. Five of the 71 Group III fetuses were malformed (Tables I and V): three had hydrocephaly, one of these also had unilateral hydronephrosis, and one had a very small left lateral ventricle. Placentas in litters with malformed fetuses were more severely damaged, including also severe yolk sac lesions, than those of litters with fetuses free of anomalies.

Moderate or severe neuronal necrosis was present in



Fig. 3. Normal yolk sac placenta. (Hematoxylin and eosin; original magnification × 260.)

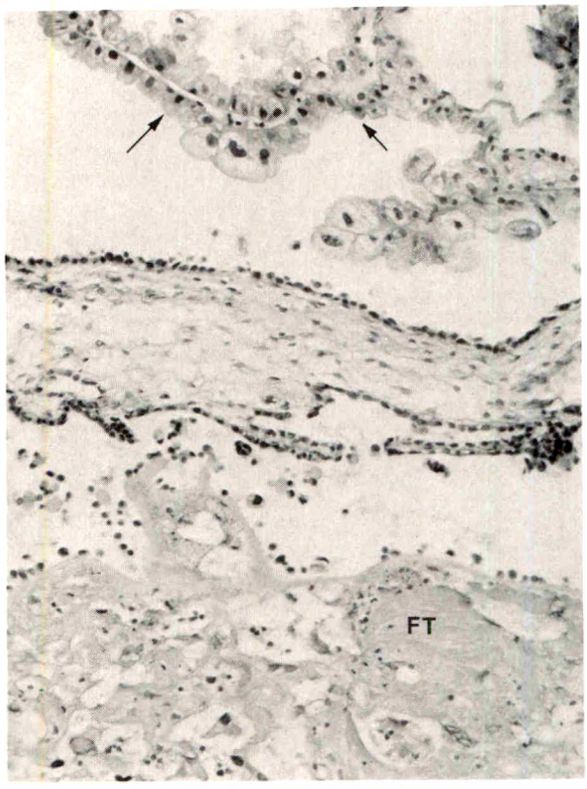


Fig. 4. Pathologic yolk sac placenta. In addition to the ballooning of the epithelial cells note random cell necrosis (arrows) and subchorionic necrosis of the labyrinth associated with a fibrin thrombus *(FT)*. (Hematoxylin and eosin; original magnification × 260.)

more than 50 per cent of the Groups II and III experimental fetuses but was relatively rare in Group I experimental fetuses and in the control (Table II, Fig. 8).

In a total of five experimental fetuses subcutaneous, abdominal, and.or thoracic hemorrhages were observed (Tables III, IV, and V).

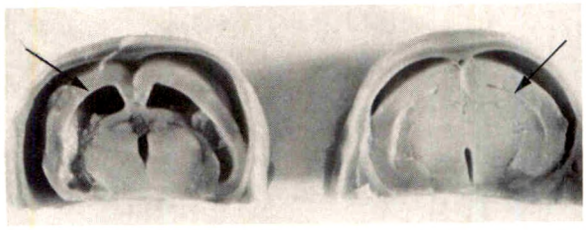


Fig. 5. Coronal sections of fetal heads. Arrows point to the lateral ventricles. Note the difference between hydrocephaly and a normal brain.

Comment

Several years have elapsed since the development of the limulus coagulation test for the detection of endotoxemia.[10] Methodologic problems still exist in the utilization of the test and, perhaps partly because of this, there has been limited clinical and laboratory study of endotoxemia in pregnancy.

In clinical neonatology placental examination is rarely used for the diagnosis and study of congenital disease[2] and in the many animal studies that have been performed with various teratogenic agents, morphologic placental changes also have seldom been evaluated. This is regrettable since, both in clinical medicine[1] and in laboratory investigation, morphologic placental studies have been shown to elucidate many aspects of the pathogenesis of congenital disease.

In the present experimental study, although we did not produce ischemic lesions in the organs of the mother or fetus, we consistently produced severe ischemic placental lesions with repeated doses of endotoxin. Treatment at the appropriate early developmental phase was productive of fetal anomalies in five of 74 Group II and in five of 71 Group III fetuses. Control rats, however, were free of fetal anomalies.

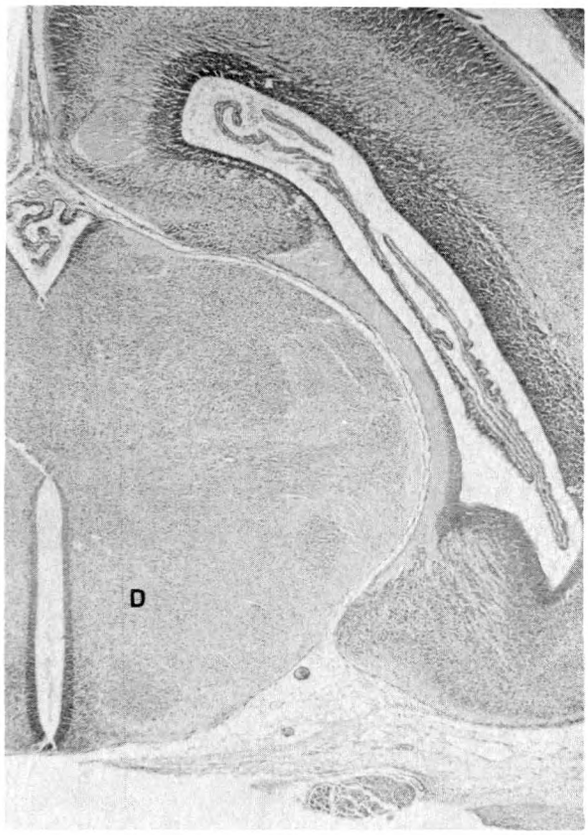


Fig. 6. Coronal section through a normal fetal brain at the level of the trigeminal ganglion. *D* = diencephalon. (Hematoxylin and eosin; original magnification × 40.)

Endotoxins are known to cause abortions in rabbits, mice, and rats [13, 21, 23] as well as in human subjects. Extensive study of endotoxin animal experimental models has been performed in the investigation of "toxemia of pregnancy"[14] but there has been a lack of application of this model to the investigation of a possible association between endotoxemia and fetal malformation.

In our experiments the rate of endotoxin production of anomalies (maximally 7 per cent) was lower than that generally obtained with many other teratogenic agents.[24] We consider that the mechanism of teratogenesis with endotoxin includes fetal hypoxia resultant from endotoxin-induced severe placental ischemia as well as possible malnutrition or metabolic and other derangements. Extensive hemorrhages have been produced in chicken and mice fetuses subjected to hypoxia.[6, 22] Thus it may be that the widespread hemorrhages in five of our experimental fetuses result from fetal hypoxia. Fetal hypoxia, a proved teratogen in many species,[6, 22] is presumably one of the major consequences of severe placental lesions. Rat embryos are relatively resistant to hypoxia, the incidence of hypoxia-induced anomalies being lower in them than in other species. [6, 24] All of our treated fetuses could be correlated at least with severe damage of the maternal floor of the placenta (Table II). Moreover, in all mothers that produced malformed fetuses, the yolk sac placenta was also severely damaged. This suggests an association between yolk sac abnormalities and fetal anomalies, an occurrence which has been demonstrated by other investigators.[24]

There is disagreement as to the pathogenesis of the placental lesions in experimental endotoxemia in terms of whether or not a Shwartzman phenomenon is operative.[7, 13, 20] Our endotoxin-treated animals always suffered ischemic necrosis of the placental floor, often with fibrin thrombi and with ischemic lesions elsewhere in the placenta. These changes are characteristic of the Shwartzman phenomenon. We consider that the placental lesions, when induced at the appropriate time of gestation, may well be the cause of some of the fetal anomalies. Ischemic placental damage has been found after administration of high doses of vitamin D_2[16] and of estrogen[17] to pregnant rats. Decreased weight and skeletal anomalies were found in the fetuses.

Our light microscopy studies provided evidence that the fetal lesions obtained were not significantly attributable to maternal or fetal infections. In two instances of Group III, however, maternal pneumonia, deciduitis, and chorioamnionitis were present. Since there were no light microscopy findings of infection in the associated fetuses we included these animals in the study. It is possible that fetal shock induced by fetal endotoxemia may cause neuronal necrosis. We did not find disseminated intravascular coagulation or light microscopy signs of fetal shock. Furthermore, a lack of intrauterine fetal growth retardation makes it difficult to explain the mechanism of the neuronal necrosis as being attributable to shock.

Because our control animals were free of morphologically significant placental lesions and of fetal anomalies, it is reasonable to infer that the pathogenesis of fetal damage included placentally mediated damage. We are aware of a great number of reports of maternal pyrexia as a teratogenic agent.[3, 4] Although in our methodology we did not measure the maternal temperature, we recognize that endotoxemia is well known to cause hyperpyrexia and that the latter causes fetal damage mainly manifest within the central nervous system and the skeleton.[3, 4] A direct transplacental effect of endotoxin may be operative. To date, however, a lack of investigations prelcudes knowledge



Fig. 7. Coronal section through a dysplastic brain at the level of the trigeminal ganglion. (Hematoxylin and eosin; original magnification × 40.)

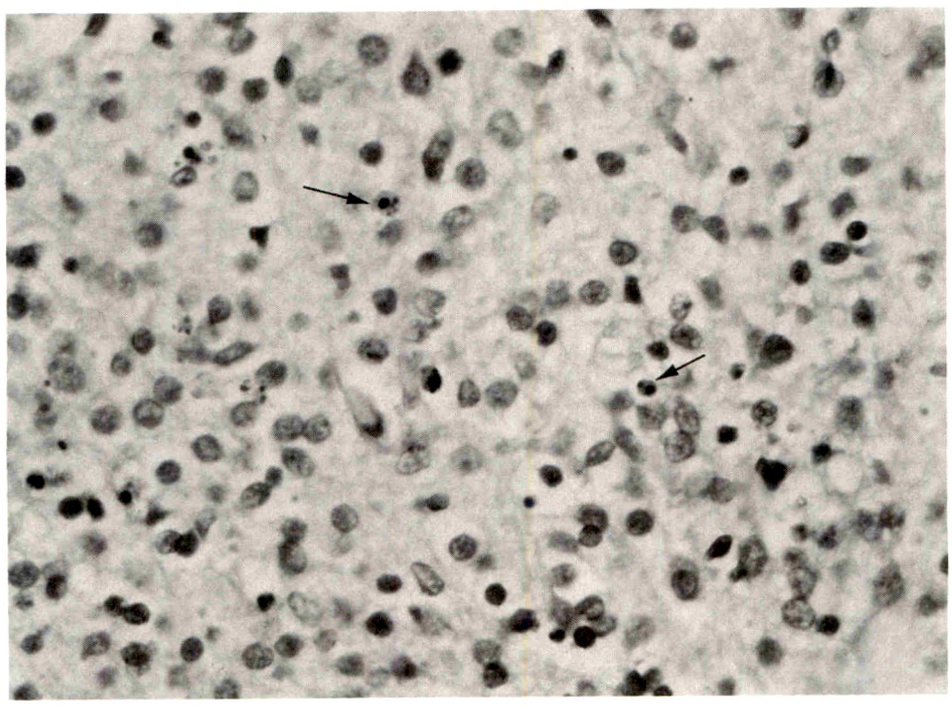


Fig. 8. Coronal section through the diencephalon. There are many necrotic neurones (arrows). (Hematoxylin and eosin; original magnification × 640.)

of whether endotoxin is able to cross the rat and the human placenta.

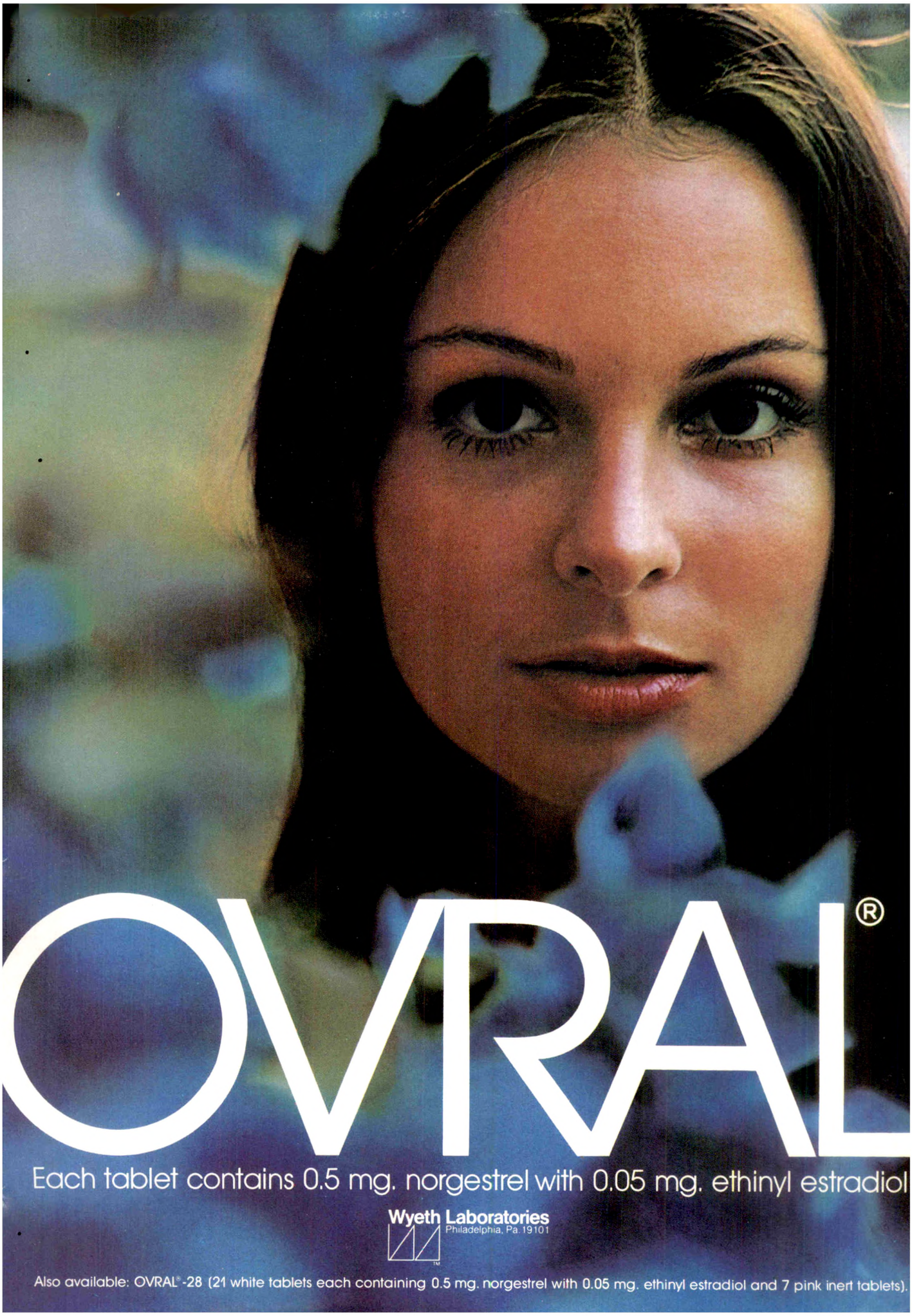
Several years ago Perrin and Landing[19] indicated that human perinatal infection, notably with gram-negative organisms, is often associated with lesions of the periventricular white matter. Gillies and associates[5] have demonstrated hydrocephalus *ex vacuo* and neonatal telencephalic leukoencephalopathy in the brains of newborn kittens treated intraperitoneally with endotoxin. Throughout our study it was apparent that there was a correlation between endotoxin treatment and fetal neuronal necrosis. A moderate to marked degree of neuronal necrosis was often found in the brains of many experimental Group II and experimental Group III fetuses but such lesions were relatively rare in the brains of control fetuses (Table II). Of additional interest is the fact that our findings of a pattern of hydrocephaly, microcephaly, brain dysplasia, microphthalmia, and anophthalmia are comparable with those of Patrick,[18] who reported "dorsal midline fusion defects" in human infants born to mothers who suffered chronic bacteriuria.

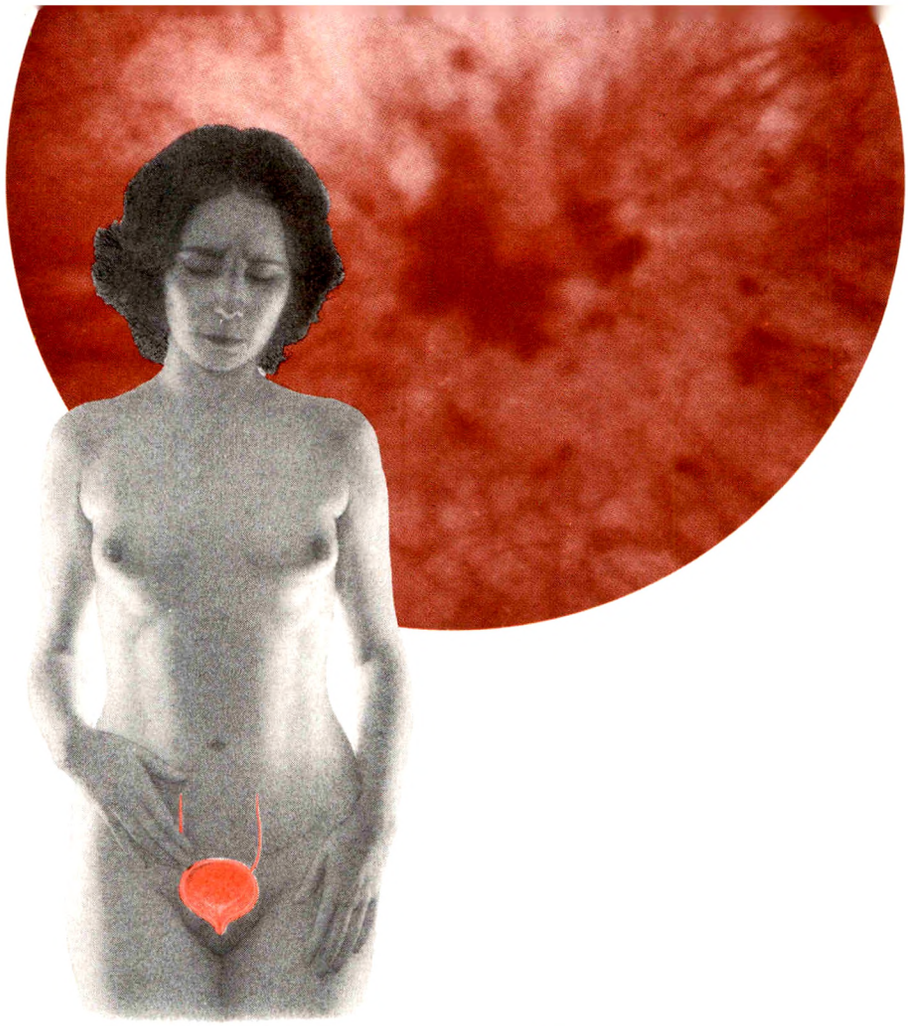
Thiersch,[23] in systematic studies of various bacterial endotoxins in pregnant rats, found placental damage but rarely any fetal anomalies. The methodology of that investigation was considerably different from ours inasmuch as the majority of the fetuses were evaluated after a few days of exposure to this insult. It is remarkable that maternal endotoxemia, a common complication of pregnancy, has been accorded such little investigation.

The authors express appreciation to Dr. Josef Warkany, their catalyst and consructive critic. Thanks are due to M. Muzzy and R. Lal for their skilled technical assistance.

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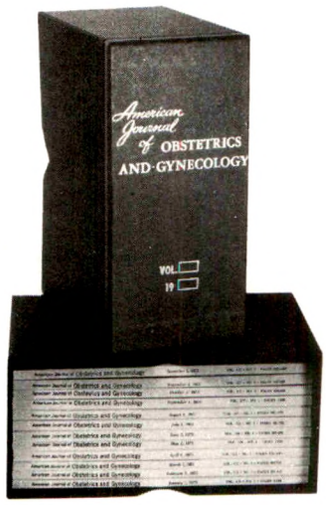
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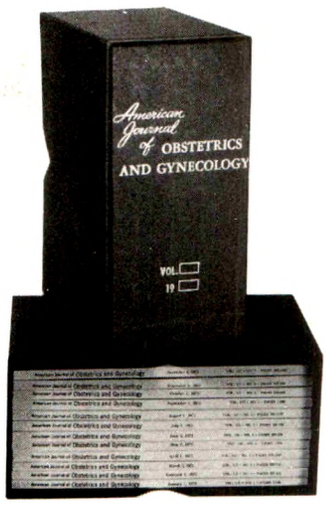
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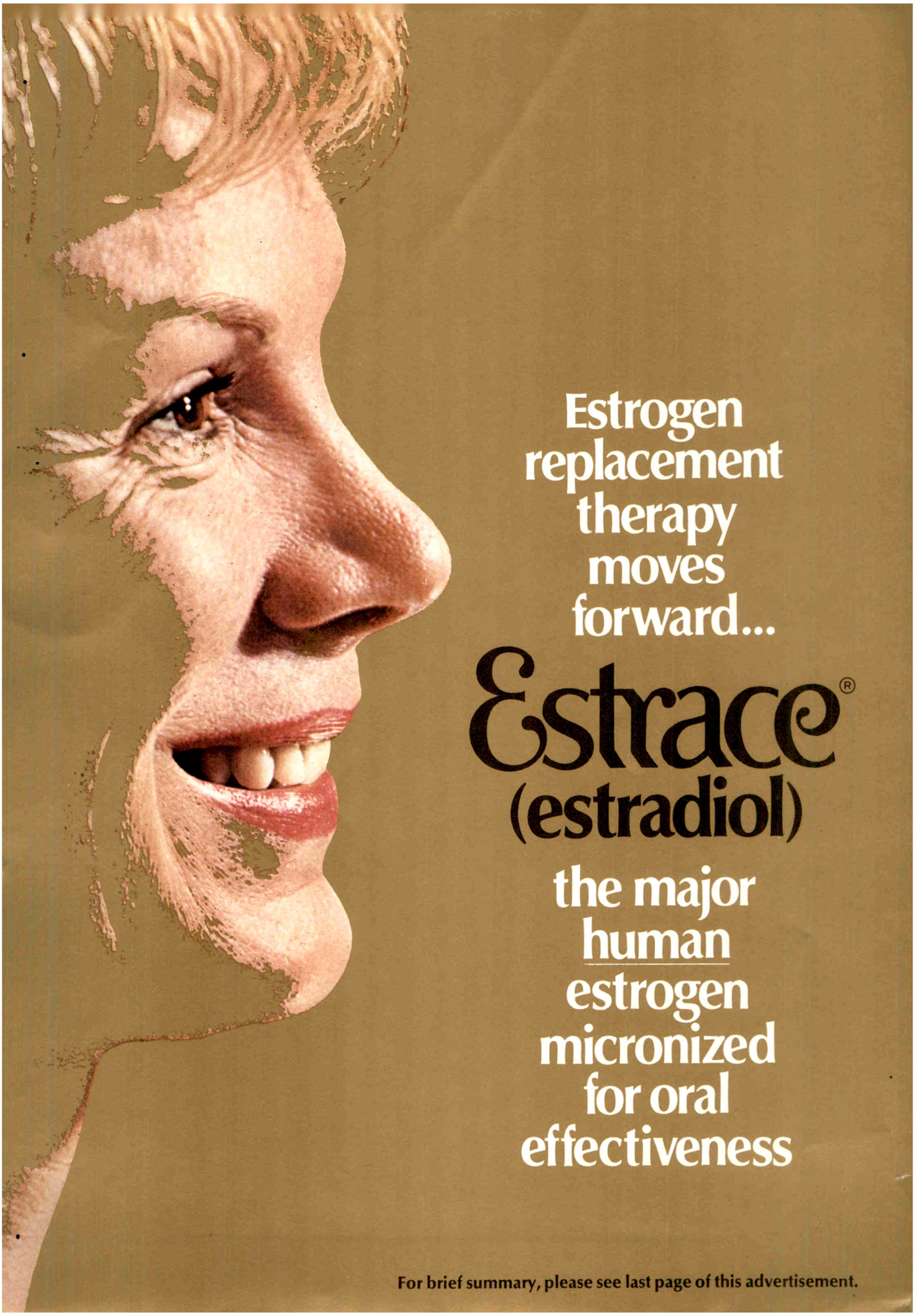
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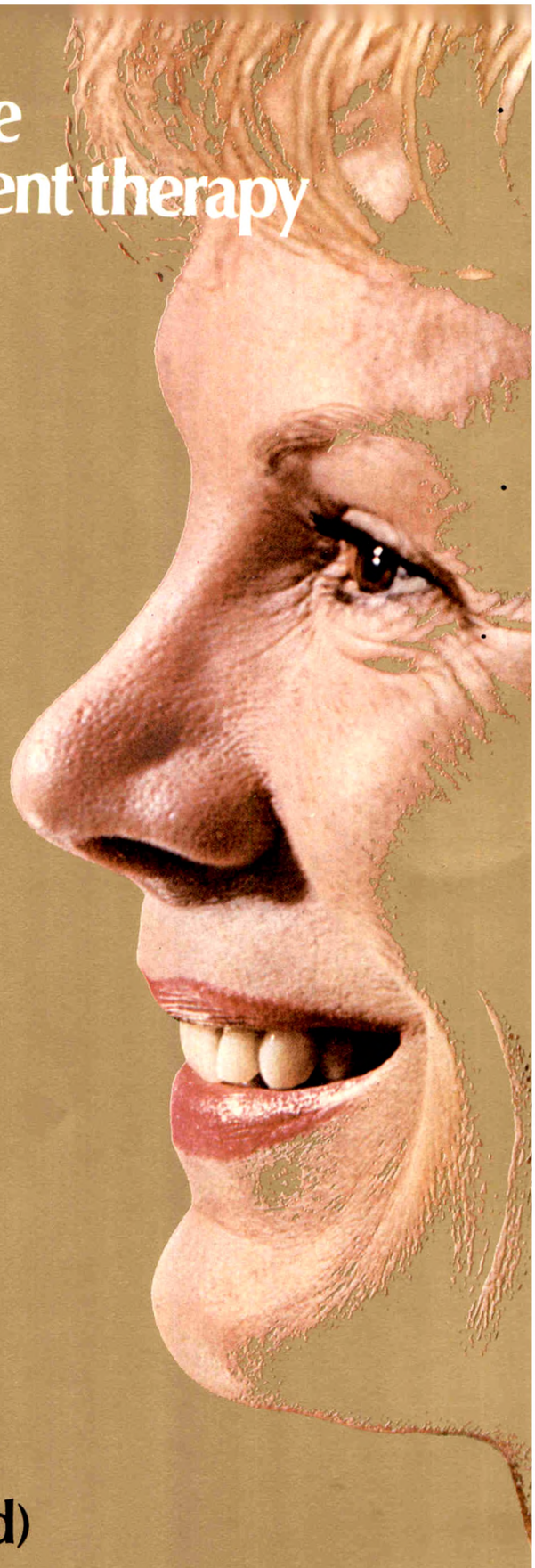
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Hyperfibrinogenemia and polycythemia with intrauterine growth retardation in fetal lambs
Loren R. Pickart, Ph.D., Robert K. Creasy, M.D., and M. Michael Thaler, M.D.
San Francisco, California


Estrogen
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moves
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Estrace®
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the major
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An important advance in estrogen replacement therapy



Mead Johnson introduces

Estrace®
(estradiol)

1-mg. and 2-mg. scored tablets

- the primary estrogen of the human ovary
- synthesized in free form (not esterified, not conjugated)

overall, 95% of patients studied gained adequate or complete relief of their symptoms[1]

Estrace (estradiol) was given to 369 estrogen-deficient women for periods ranging up to a year or more (average of six months); 319 of these women received an initial dosage of 1 or 2 mg. per day and were evaluated for efficacy. *Complete disappearance* of hot flushes was experienced by 227 (76%) of 298 women, of sweating by 131 (80%) of 164 women, of tingling by 62 (94%) of 66 women, and of genital atrophy by 44 (88%) of 50 women. In general, 95% of the patients evaluated experienced either adequate or complete relief of their symptoms. Remarkably, in 77% of the patients, symptoms were controlled without need to adjust the initial dosage—usually 2 mg. daily. (Dosage after adjustment ranged from 1 mg. to 3 or 4 mg. a day.)[1]

estradiol frequently desired

Physicians for years have desired to use estradiol, the major human ovarian hormone, as the basis for oral estrogen replacement therapy. Since 1953, several studies have reported that oral administration of estradiol, in 1 to 2 mg. doses, provided varying degrees of relief of menopausal symptoms.[2–4] One study[4] showed that the optimum estrogenic effect was obtained with 2 mg. daily.

In a preliminary investigation, Martin, *et al.*,[5] 1972, reported on a single-blind study of 71 women given four different estrogenic preparations. A formulation of micronized estradiol was selected by more women than any other preparation. In fact, twice as many selected micronized estradiol than conjugated equine estrogen.* Yen, *et al.*,[6] showed that micronized estradiol (Estrace®) given orally is readily absorbed, metabolized in part to estrone, and is biologically active.

With micronized estradiol there is no need for special coatings which may hamper tablet absorption and no "barnyard" aftertaste—problems sometimes seen with conjugated equine estrogens.

*Because the micronized estradiol formulations may differ, these data are not necessarily applicable to Estrace.

estradiol... orally effective

The technique of micronization has opened up the oral route to natural human estrogen replacement with Estrace. Reduced particle size of the estradiol in Estrace makes effective gastrointestinal absorption possible. Onset of action is rapid, with marked increases in levels of circulating estradiol and estrone; concomitant decreases in serum concentrations of FSH and LH are sustained over a 24-hour period.[6]

Estrace apparently creates a reservoir of physiologically active estrogen that is available to the cells on an "as needed" basis.[6] And because estradiol can be assayed in plasma, Estrace can provide objective[6] as well as clinical evidence[1] of ready availability and biologic activity.

well accepted by patients[1]

In the 369 patients previously described, the incidence of estrogen-induced side effects with Estrace was relatively low. Incidence of nausea was remarkably low—only 2% of patients before dosage adjustment. Some patients who had complained of this problem while on conjugated equine estrogens reported its complete disappearance on changing to Estrace. Whereas before dosage adjustment 24% experienced breast tenderness, 31% edema, 18% (of 151 with intact uteri) bleeding, and 4% weight gain, only about 2% of the 369 patients dropped out because of side effects.[1]

A few instances of significant gynecologic disease became apparent during clinical investigations.[1] Endometrial hyperplasia was discovered in seven patients, all of whom had previously received estrogen therapy for one to three years prior to entering the study. One patient was reported to have carcinoma *in situ* and another showed adenocarcinoma of the endometrium. In clinical practice, the physician should be alerted to the potential of thrombotic disorders and be prepared to discontinue medication immediately if they occur. Because of variations in normal endogenous hormone production, certain patients may exhibit signs of excessive estrogenic stimulation. Adverse reactions generally associated with estrogen administration may occur and these are listed in the brief summary. Prior to initiation of treatment with Estrace or any other estrogenic preparation, an adequate physical examination should be given.

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For Brief Summary, please see following page.

Estrogen replacement therapy moves forward...



Estrace® (estradiol)

the major human estrogen micronized for oral effectiveness

Indications: Estrace® (17β-Estradiol) is indicated for relief of endogenous estrogen deficiency and associated menopausal symptoms.

Contraindications: Estrace® is contraindicated in patients with 1) markedly impaired liver function; 2) known or suspected carcinoma of the breast, except those cases of progressing disease not amenable to surgery or irradiation occurring in women who are at least 5 years postmenopausal, or in men in whom castration is not feasible; 3) known or suspected estrogen dependent neoplasia, such as carcinoma of the endometrium; 4) undiagnosed abnormal genital bleeding; 5) thrombophlebitis, thromboembolic disorders, cerebral apoplexy, or with a past history of these conditions; and 6) pregnancy.

Warnings: The physician should be alert to the earliest manifestations of thrombotic disorders (thrombophlebitis, cerebrovascular disorders, pulmonary embolism and retinal thrombosis). If these occur or are suspected the drugs should be discontinued immediately. Discontinue medication pending examination if there is a sudden onset of proptosis, diplopia, or migraine. If examination reveals papilledema or retinal vascular lesions, medication should be withdrawn. Estrogens may be excreted in the mother's milk and an estrogenic effect upon the infant has been described. The long range effect on the nursing infant cannot be determined at this time. Hypercalcemia may occur in as many as 15% of breast cancer patients with metastases and this usually indicates progression of bone metastases. This occurrence depends neither on dose nor on immobilization. In the presence of untoward effects such as progression of the cancer or hypercalcemia, estrogen medication should be stopped. A statistically significant association has been reported between maternal ingestion during pregnancy of diethylstilbestrol and the occurrence of vaginal carcinoma developing years later in the offspring. Whether such an association is applicable to all estrogens is not known at this time. In any event, estrogens are not indicated for use during pregnancy.

Precautions: Because normal endogenous hormone production varies individually, certain patients may be unusually responsive to estrogenic therapy and may respond with undesirable manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding, mastodynia, edema, etc. Because of estrogen induced salt and water retention, these drugs should be used with caution in patients with epilepsy, migraine, asthma, cardiac or renal disease. Patients with a history of psychic depression should be carefully observed and the drug discontinued if the depression recurs to a serious degree. In the event that any unexplained or excessive vaginal bleeding would occur while on estrogen therapy, nonfunctional causes should be borne in mind. The drug should be discontinued and a thorough investigation made as to the cause, being certain to rule out the possibility of malignancy. Pre-existing uterine fibromyomata may increase in size while using this product, therefore, patients should be examined at regular intervals while receiving estrogenic therapy. Women with a strong family history of cancer, recurrent chronic cystic mastitis, or abnormal mammograms should be administered estrogens with caution. Because of a possible decrease in glucose tolerance, diabetic patients should be followed closely. Because estrogens influence the metabolism of calcium and phosphorus, they should be used with caution in patients with certain metabolic bone diseases that are associated with hypercalcemia or in patients with renal insufficiency. The pathologist should be advised of estrogen therapy when relevant specimens are submitted. Because of the effects of estrogens on epiphyseal closure, they should be used judiciously in young patients in whom bone growth is not complete. A pre-treatment physical examination should include special reference to breast and pelvic organs as well as a Papanicolaou smear since estrogens have been known to produce tumors, some of them malignant, in five species of animals. When large doses of estrogens are used, urinary stress incontinence may occur in nonpregnant females. Prolonged high doses of estrogens will inhibit anterior pituitary functions. This should be borne in mind when treating patients in whom fertility is desired. Continuous use of estrogens will result in prolonged stimulation of the endometrium and breast. In order to avoid this, oral estrogens should be administered cyclically in the menopausal or hypogonadal patient. The age of the patient constitutes no absolute limiting factor, although treatment with estrogens may mask the onset of the climacteric. Certain endocrine and liver function tests may be affected by treatment with estrogens. If such tests are abnormal in a patient taking these drugs it is recommended that they be repeated after the drug has been withdrawn for two months. Any possible influence of prolonged estrogen therapy on pituitary, ovarian, adrenal, hepatic, or uterine function awaits further study.

Adverse Reactions: A statistically significant association has been demonstrated between use of estrogen-progestin oral contraceptives and the following serious reactions: Thrombophlebitis, pulmonary embolism, and cerebral thrombosis. Although available evidence is suggestive of an association, such a relationship has been neither confirmed nor refuted for the following serious reactions: coronary thrombosis and neuro-ocular lesions (e.g. retinal thrombosis and optic neuritis). The following adverse reactions are known to occur in patients receiving estrogens: nausea, vomiting, anorexia, gastrointestinal symptoms (such as abdominal cramps or bloating), edema, breakthrough bleeding, spotting or withdrawal bleeding, breast tenderness and enlargement, change in body weight (increase or decrease), headache, increased cervical mucus, allergic rash, loss of libido and gynecomastia in the male, reactivation of endometriosis, aggravation of migraine headaches, hepatic cutaneous porphyria becoming manifest, cholestatic jaundice, rise in blood pressure in susceptible individuals, mental depression, cystitis-like syndrome, loss of scalp hair, erythema nodosum, hemorrhagic eruption, premenstrual-like syndrome, changes in libido, changes in appetite, nervousness, dizziness, fatigue, backache, erythema multiforme, itching, possible diminution in lactation when given immediately post-partum, irritability, and malaise.

Important Notes: An increased risk of thromboembolic disease associated with the use of oral contraceptives containing estrogens and progestins has now been conclusively established. Retrospective studies have shown a statistically significant association between thrombophlebitis, pulmonary embolism, and cerebral thrombosis and embolism and the use of these drugs. There have been three principal studies in Great Britain[1-3] and one in the United States[4] leading to this conclusion. As a result of these studies, it has been estimated that users of oral contraceptives containing estrogens are 4 to 7 times more likely than nonusers to develop thromboembolic disease without evident cause. The American study also indicated that the increased risk did not persist after discontinuation, nor was it enhanced by long continued administration. Although the American study was not designed to evaluate a difference between products, it did suggest that there might be an increased risk of thromboembolic disease in users of sequential products. Confirmation of this finding requires further study. In a more recent analysis of data derived from several national adverse reaction reporting systems,[5] British investigators concluded that the risk of thromboembolism, including coronary thrombosis, is directly related to the dose of estrogen used in oral contraceptive products. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. Nevertheless, in view of this study, as well as others that have demonstrated a positive relationship between estrogens and thromboembolism, it would seem prudent and in keeping with basic therapeutic principles, to utilize, whenever feasible, the smallest effective dose of estrogen in treating patients. Risks associated with certain other known adverse reactions, such as elevated blood pressure, liver dysfunction, and reduced tolerance to carbohydrate, have not as yet been quantitated. Long term administration of both natural and synthetic estrogens in subprimate animal species in multiples of the human dose increases the frequency for some animal carcinomas. These data cannot be transposed directly to man. The possible carcinogenicity due to the estrogens can neither be confirmed nor refuted at this time. Close clinical surveillance of all women taking estrogens must be continued. It has been reported in a recent study done in the United States[6] that the maternal ingestion of diethylstilbestrol during pregnancy appears to increase the risk of vaginal adenocarcinoma developing years later in the offspring exposed.

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Dosage and Administration: Treatment is usually initiated with a dose of 1 or 2 mg. daily of micronized estradiol. Dosage may be adjusted to control the presenting symptoms depending on the response of the individual patient; the minimal effective dose for maintenance therapy should be determined by titration. To avoid continuous stimulation of the reproductive tract and breasts, cyclic therapy is recommended. The usual regimen consists of three weeks on drug followed by one week off drug. Some patients may require a slightly shorter estrogen-free interval (4-6 days) to prevent recurrence of menopausal symptoms. Withdrawal bleeding of unusual amount or duration should be investigated.

How Supplied: Estrace® 1 mg; lavender scored tablets, NDC 0087-0755-01, bottles of 100. Estrace® 2 mg; turquoise scored tablets, NDC 0087-0756-01, bottles of 100.

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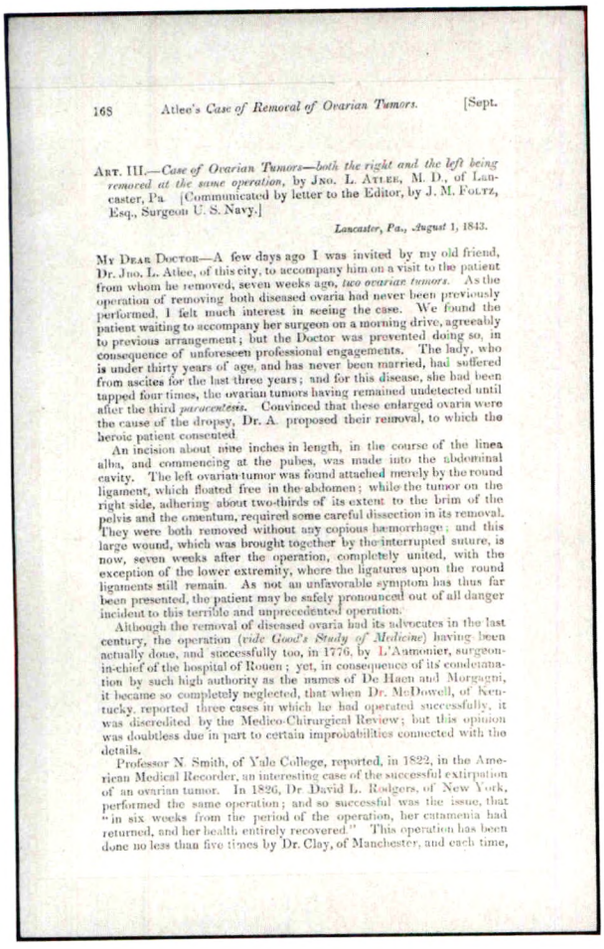
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Classic pages in
OBSTETRICS AND GYNECOLOGY
Lawrence D. Longo, M.D.

Case of ovarian tumors—both the right and the left being removed at the same operation

JOHN LIGHT ATLEE

The New York Journal of Medicine and the Collateral Sciences, vol. 1, pp. 168-170, 1843



168 Atlee's *Case of Removal of Ovarian Tumors.* [Sept.

ART. III.—*Case of Ovarian Tumors—both the right and the left being removed at the same operation*, by JNO. L. ATLEE, M. D., of Lancaster, Pa. [Communicated by letter to the Editor, by J. M. FOLTZ, Esq., Surgeon U. S. Navy.]

Lancaster, Pa., August 1, 1843.

MY DEAR DOCTOR—A few days ago I was invited by my old friend, Dr. JNO. L. Atlee, of this city, to accompany him on a visit to the patient from whom he removed, seven weeks ago, *two ovarian tumors*. As the operation of removing both diseased ovaria had never been previously performed, I felt much interest in seeing the case. We found the patient waiting to accompany her surgeon on a morning drive, agreeably to previous arrangement; but the Doctor was prevented doing so, in consequence of unforeseen professional engagements. The lady, who is under thirty years of age, and has never been married, had suffered from ascites for the last three years; and for this disease, she had been tapped four times, the ovarian tumors having remained undetected until after the third *paracentesis*. Convinced that these enlarged ovaria were the cause of the dropsy, Dr. A. proposed their removal, to which the heroic patient consented.

An incision about nine inches in length, in the course of the linea alba, and commencing at the pubes, was made into the abdominal cavity. The left ovarian tumor was found attached merely by the round ligament, which floated free in the abdomen; while the tumor on the right side, adhering about two-thirds of its extent to the brim of the pelvis and the omentum, required some careful dissection in its removal. They were both removed without any copious hæmorrhage; and this large wound, which was brought together by the interrupted suture, is now, seven weeks after the operation, completely united, with the exception of the lower extremity, where the ligatures upon the round ligaments still remain. As not an unfavorable symptom has thus far been presented, the patient may be safely pronounced out of all danger incident to this terrible and unprecedented operation.

Although the removal of diseased ovaria had its advocates in the last century, the operation (*vide Good's Study of Medicine*) having been actually done, and successfully too, in 1776, by L'Aumonier, surgeon-in-chief of the hospital of Rouen; yet, in consequence of its condemnation by such high authority as the names of De Haen and Morgagni, it became so completely neglected, that when Dr. McDowell, of Kentucky, reported three cases in which he had operated successfully, it was discredited by the Medico-Chirurgical Review; but this opinion was doubtless due in part to certain improbabilities connected with the details.

Professor N. Smith, of Yale College, reported, in 1822, in the American Medical Recorder, an interesting case of the successful extirpation of an ovarian tumor. In 1826, Dr. David L. Rodgers, of New York, performed the same operation; and so successful was the issue, that "in six weeks from the period of the operation, her catamenia had returned, and her health entirely recovered." This operation has been done no less than five times by Dr. Clay, of Manchester, and each time,

John L. Atlee of Lancaster, Pennsylvania, revived the operation of ovariotomy, pioneered by Ephraim McDowell. This, one of his earliest cases, was the first successful removal of both ovaries, one of which weighed 1¼ pound and the other weighed ¾ pound. Following a historical account of the operation of oophorectomy, Atlee wrote, "I have thus briefly summed up a history of the operations for diseased ovaria, under the belief that these cases, combined, tend to establish a new principle in surgery, viz, the comparative safety of making large openings into the cavity of the abdomen. . . . The successful result, generally speaking, attending the extirpation of enlarged ovaria, would seem, however, to show that operations within the abdomen are, by no means, so fatal as the profession have been led to believe. . . ." From 1843 to 1883, he performed oophorectomy about 78 times with 64 recoveries. This mortality rate of only 18 per cent was remarkable for its time, but by the end of the century the mortality rate for the operation had decreased to 1 per cent. In addition to having a busy general practice, Atlee served as professor of anatomy and physiology at Franklin and Marshall College and was one of the founders of the American Medical Association.

COMMUNICATIONS IN BRIEF

This section is suitable for reporting cases, results of therapeutic trials, and descriptions of new procedures or instruments. Reports should be limited to seven hundred words and two references. Use of an illustration or table requires a proportionate reduction in total words.

Peritoneal-cutaneous fistula secondary to a perforated Dalkon shield

LAURA SLAUGHTER, M.D.
DAVID J. MORRIS, M.D.
Women's Hospital, Los Angeles, California

THE FOLLOWING case report is presented as an unusual manifestation of a perforated Dalkon shield.

M. B., A 25-year-old black woman, gravida 5, para 4, therapeutic abortion 1, had a Dalkon shield inserted 6 weeks post partum in June, 1972. The insertion was extremely painful and she requested immediate removal but agreed to a 3 week trial period. During this interval she experienced spotting and cramping which subsided completely by her next appointment. At that time, she was informed after routine pelvic examination that the device was no longer present and she was given oral contraceptives. In the interim prior to admission she had an abortion in January, 1973, was treated for gonococcal salpingitis on August 14, 1973, and had at least four documented visits to a family-planning clinic for birth control pills. On September 21, 1974, she was seen in the emergency room because a 3 by 3 cm. infraumbilical mass which had been present for over a year had suddenly become painful and drained purulent material. A 1 cm. abscess was incised and drained; bacteroides was cultured from the abscess site. Despite these measures, the abscess continued to drain and she was seen on three subsequent occasions. Finally, on December 2, 1974, the abscess cavity, which was now 2 by 2 cm., yielded a black, single-knotted string. A flat plate and lateral x-ray disclosed a Dalkon shield just beneath the abdominal wall. At surgery, the Dalkon shield was found encircled by omentum and fibrous tissue in a 4 x 3 cm. mass. The string led from the peritoneal cavity through the abdominal wall to just beneath the skin and is represented as a radiolucent tract on lateral x-ray (see Fig. 1). The abscess was confined to the abdominal wall.

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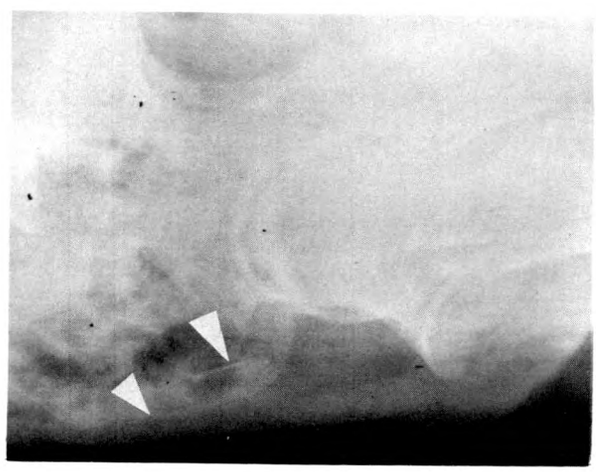


Fig. 1. Lateral x-ray.

Whitson, Israel, and Bernstein,[1] in a recent report on intrauterine Dalkon shields, noted that the majority of cases occurred in multiparas with a standard size device in the puerperium and were associated with a significant chronic adhesive reaction necessitating removal. They carefully outlined a workup for the localization of IUD's suspected to have perforated. This case and others reporting multiple IUD insertions[2] emphasize to the clinician that the absence of IUD strings must not be assumed to represent expulsion per vagina and demands further investigation to rule out perforation.

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Maternal midtrimester sepsis in association with the intrauterine contraceptive device: Early histopathologic findings

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MATERNAL midtrimester sepsis in association with the shield-type intrauterine contraceptive device (IUD) has now been reported by several authors.[1-4] The mechanisms by which this infection occurs and is disseminated are unknown. The clinical presentation is unusual in that there are often symptoms of generalized infection, a "flu-like" syndrome, with absent or mild symptoms and signs of localized pelvic infection. The case reported here is similar to those previously described, but is presented because of the somewhat unusual microscopic findings.

A 24-year-old white woman, gravida 1, para 0, ABO, presented at 16 weeks' gestation with a shield-type IUD in place and a 12 hour history of chills and fever. She had no other complaints. General physical examination revealed a temperature of 104.6° F. rectally, a blood pressure of 110/60 mm. Hg and a pulse of 120, but was otherwise unremarkable. Pelvic examination confirmed a 14 to 16 week size intrauterine pregnancy. The uterus was mildly tender, and the fetal heart tones were 200 b.p.m. After appropriate laboratory studies and bacterial cultures were obtained, therapy with antibiotics and pitocin was begun.

The fetus, placenta, and IUD were passed vaginally and a curettage was performed. Postoperatively, the patient responded rapidly to continued antibiotic therapy and there were no complications. Maternal blood and placental cultures grew *E. coli*.

Histopathologic examination of the specimen revealed extensive bacterial colonization on the maternal side of the membranes (Fig. 1). Microscopic sections of the placenta and fetal organs were unremarkable.

The mechanism by which severe maternal sepsis occurs in association with an intrauterine pregnancy and a shield-type IUD has been speculated on by the authors reporting previous cases. Because of the rapid clinical deterioration due to septicemia when signs of severe localized pelvic infection were often absent, Christian[1] felt that there might be a mechanical factor which allowed vascular dissemination of bacteria at a time when the infection might be otherwise locally contained. In those cases in which the microscopic examination of the pregnancy has been described, well-established chorioamnionitis and placentitis were most often noted.

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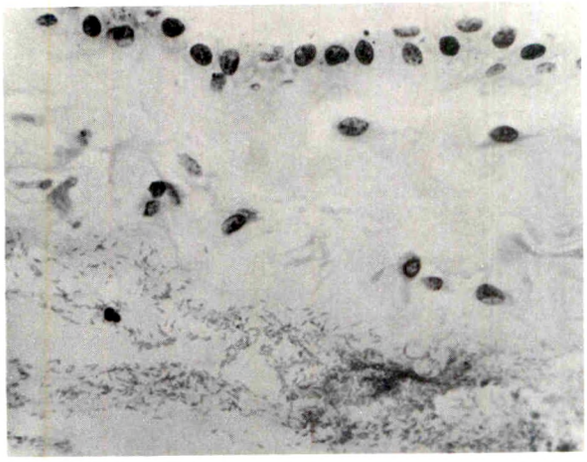


Fig. 1. Amnion, demonstrating epithelial surface (above) and numerous gram-negative bacilli (below). A single polymorphonuclear leukocyte is present. (Hematoxylin and Eosin; ×416).

We believe that the case reported here represents an earlier example of this clinical problem than those generally reported. The finding of large numbers of bacteria on the maternal side of the membranes, without amnionitis or placentitis, is unusual. One might speculate that this could explain the dissemination of bacteria into the maternal vascular system, resulting in severe maternal septicemia prior to the development of the classical signs and symptoms of septic abortion. This is only speculation and in no way attempts to explain how this phenomenon occurs with the IUD. We feel, however, that the histologic findings in this case are consistent with the clinical course seen in this patient and perhaps of other similar reported cases.

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January 15, 1976
Am. J. Obstet. Gynecol.

Gonadal dysgenesis in two siblings

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MELVIN BERLIN, M.D., F.A.C.O.G.
MILTON M. DANA, M.D., F.C.A.P.

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ONE OF THE most fascinating and yet poorly handled entities in medicine is gonadal dysgenesis. X-Y gonadal dysgenesis presents the clinician with a complete challenge. Embryology, genetics, anatomy, endocrinology, physiology, pharmacology, and psychology are all intimately necessary in diagnosing and treating this disorder. In order to recognize the dysgenetic patient we must have a high suspicion level with each case of amenorrhea.

The complete absence of a functioning gonad always, and irrespective of chromosomal pattern, results in the development of the müllerian duct system. This was shown by the experimental work of Jost. By removing the fetal gonads of mice at an early stage in intrauterine life, Jost produced animals with female genitalia regardless of the gonadal sex.

We are now finding more cases of gonadal dysgenesis appearing in the medical literature with increasing evidence that X-Y gonadal dysgenesis is an X-linked recessive or male-limited autosomal dominant condition. This paper adds two cases of pure gonadal dysgenesis in siblings.

Case 1. A 15-year-old phenotypic female was first seen for amenorrhea. She was the product of a 9 month gestation with a birth weight of 5 pounds, 5 ounces. There were two siblings, one older sister with amenorrhea and a normal younger brother. The patient's height was 63 inches, arm span 62 inches, pubis-to-floor measurement 32 inches, weight 133 pounds, blood pressure 130/70 mm. Hg. There was no suggestion of breast development and no axillary or pubic hair. The labia, clitoris, and vagina were normal, with an infantile uterus and cervix. Firm band-like structures were palpated in place of adnexal tissue on careful bimanual examination.

A buccal smear yielded two Barr bodies per 100; human pituitary gonadotropin 96 μ; intravenous pyelogram, negative. 17-OH and ketosteroids were normal. Karyotyping of peripheral blood lymphocytes demonstrated a 46 X-Y pattern and no evidence of mosaicism with examination of 20 metaphase figures.

At laparotomy a small infantile uterus with normal-appearing tubes were found. Two small, white, fibrous, streak gonads were removed. The patient is presently being cycled with Premarin 1.25 mg. and Provera 10 mg., with satisfactory breast development and the realization that she will develop as a healthy female but will be unable to bear offspring unless ovarian transplants become available.

Reprint requests: Dr. Milton M. Dana, Director of Laboratories, Southside Hospital Montauk Highway, Bayshore, New York 11706.

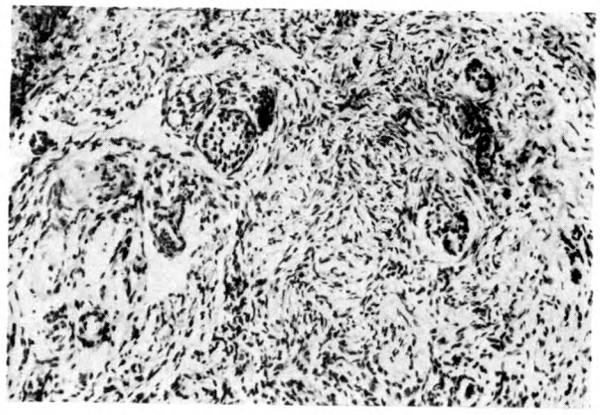


Fig. 1. Case 1. Undifferentiated gonadal tissue with epithelial inclusions. (Hematoxylin and eosin; ×200.)

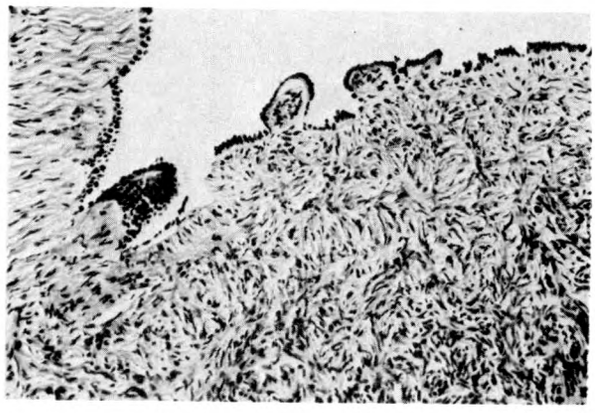


Fig. 2. Case 2. Compact and undifferentiated gonadal tissue partially covered by surface epithelium. (Hematoxylin and eosin; ×200.)

Histologic examination of the removed gonads revealed a compact fibrous stroma devoid of ovarian follicles or seminiferous tubules. Rare groups of Leydig-like cells and a few small celomic-type epithelial inclusions were seen (Fig. 1).

Case 2. The 23-year-old sibling of the patient described in Case 1 presented herself at the office after her sister had surgery. She had initially been seen at age 15 for amenorrhea and her condition was diagnosed as "Turner's agenesis." She was taking estrogens and progestens to produce cyclic bleeding and was told that she might have children if given "fertility pills."

Physically, she is an attractive, large-boned, thin, phenotypic female, 64 inches tall. Her external genitals were female, with sparse axillary and pubic hair and breasts of small estrogenized type. Her vagina was 8 cm. and the uterus and cervix were of small adolescent type.

In place of adnexa, firm streak-like areas were palpated bilaterally. 17-OH and ketosteroids were normal. A buccal smear yielded one to two Barr bodies per 100 cells. An IVP was normal. Karyotyping of peripheral blood lymphocytes

demonstrated a 46 X-Y pattern and no evidence of mosaicism with examination of 20 metaphase figures.

Histologic examination of the removed gonads revealed a compact fibrous stroma devoid of any epithelial structures (Fig. 2).

Siblings with pure gonadal dysgenesis are presented, typically appearing with a history of primary amenorrhea. The diagnosis was established in the younger of the siblings initially, thus stressing the importance of cytogenetic studies in all cases of primary amenorrhea. The reported significant familial incidence of dysgenetic gonads suggests a need to study all siblings. Once the X-Y pattern is found, the experience as reported in the literature to date makes the decision for treatment relatively easy, i.e., removal of all streak gonads once the presence of a Y chromosome is established.[1, 2] Fortunately, no tumors were found in our patients. The uncertainty of sexual identification exists with the older of the two siblings because of misinformation afforded the patient initially.

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Pregnancy following 29 cycles of clomiphene citrate therapy: A case report

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DESPITE extensive experience with clomiphene citrate (Clomid), controversy remains regarding dosage, duration of therapy, and the use of alternative or additional methods of treatment. The product information brochure limits its use to dosages no greater than 100 mg. per day for 5 days and for no more than three ovulatory cycles.

This paper reports the successful induction of ovulation followed by pregnancy after 29 courses of treatment with clomiphene citrate.

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U. H., a 26-year-old nulligravida, was referred to the Endocrine-Infertility Clinic in September, 1970, because of infertility for 6 years and oligomenorrhea since her menarche at age 12. Contraceptives had never been used. She had gained 40 pounds in 7 months and was amenorrheic since February, 1970. In July, 1970, she underwent an appendectomy with lysis of multiple blood-tinged, intra-abdominal perirectal cysts. The uterus, tubes, and ovaries were described as normal.

Complete physical examination was within normal limits except for obesity. She was neither hirsute nor virilized. CBC, electrolytes, renal and liver function tests, FBS, 2 hour PP, T_3, T_4, VDRL, urinalysis, cervical cytology, and chest x-ray were within normal limits.

Clomiphene citrate, 50 mg. daily for 5 days, was administered on the fifth day of intramuscular progesterone–induced bleeding. A serum progesterone level 17 days after the last clomiphene tablet was 4.0 ng. per milliliter, consistent with ovulation. A fractional postcoital test in a subsequent cycle was normal. Hysterosalpingography and diagnostic laparoscopy with chromopertubation were also normal.

The same clomiphene dose resulted in presumably ovulatory cycles on 13 successive occasions. Treatment was interrupted for 1 month when asymptomatic, 6 cm., bilateral, ovarian cysts developed. These cysts spontaneously resolved before treatment was resumed. Despite clomiphene therapy, ovulation did not occur in February, 1972. Withdrawal bleeding was again induced and clomiphene citrate, 100 mg. per day for 5 days, was prescribed, resulting in presumptive ovulation. In November the 100 mg. dose of clomiphene failed to induce ovulation and therapy consisting of 150 mg. per day for 5 days was begun. Hematologic studies, electrolytes, and liver and renal function tests remained normal throughout clomiphene therapy. Each of 12 cycles at this dosage was ovulatory and conception occurred in February, 1974.

The pregnancy progressed uneventfully until August 26, 1974, when premature labor began. She spontaneously gave birth to a 3-pound, 9 ounce male infant, whose subsequent growth and development have been normal.

Clomiphene citrate, gonadotropins, and bilateral ovarian wedge resection are the measures used most commonly to treat anovulatory infertility. Because of complications, expense, inconvenience, and a high incidence of multiple gestations, gonadotropin therapy is warranted only in a highly selected group of patients and must be accompanied by daily monitoring of estrogen levels.

Goldzieher[1] summarized the results of wedge resection in 1,079 cases collected from 187 references: postoperatively, regular menstrual cycles occurred in six to 95 per cent (mean 80 per cent) of patients and pregnancy in 13 to 89 per cent (mean 63 per cent). The widely divergent results are secondary to patient selection, the presence of additional infertility factors, variations in surgical technique, and occasional intra- and postoperative complications.

In a recent report from our clinic in which an individualized, graduated, treatment regimen of clomiphene citrate was used to induce ovulation until pregnancy occurred, there was an ovulation rate of 91.4 per cent and an over-all pregnancy rate of 38.1 per cent.[2] When patients with other infertility factors were eliminated, however, the corrected pregnancy

rate was found to be 85.7 per cent. It is our belief that clomiphene citrate therapy is preferable to treatment by wedge resection because: (1) a major operative procedure is avoided, (2) peritubal and periovarian adhesions frequently occur after wedge resection, and (3) the recurrence of anovulatory cycles following wedge resection is common.

When clomiphene citrate successfully corrects the ovulatory defect, but pregnancy does not occur, two alternative methods of treatment have been recommended: combined clomiphene citrate–gonadotropin therapy and bilateral ovarian wedge resection. The former adds the risks of gonadotropin therapy and the latter substitutes potentially hazardous surgical therapy. The pregnancy rate following ovulation induced by these two methods has not been shown to be greater than that following ovulation induced by clomiphene citrate.

The limitation of clomiphene citrate to an arbitrary number of apparently ovulatory cycles is not warranted in the absence of other infertility factors. If a patient ovulates, but does not conceive, cyclic clomiphene treatment should be continued. In this way patients may become pregnant with greater safety than after having a laparotomy and removal of functional ovarian tissue. As illustrated by this case report, when clomiphene citrate successfully induces ovulation, treatment should be continued until conception occurs. In this patient, treatment for more than 2 years had no deleterious effects and was followed by a successful pregnancy.

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Ovarian malignancy subsequent to a Le Fort operation

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THE LE FORT colpocleisis is well known to gynecologists as a reliable procedure for the surgical repair of uterine prolapse. Although the operation has been modified in several ways, the technique includes the denuding of rectangular areas of similar size on the anterior and posterior walls of the vagina and suturing these mucosal edges together and opposing the denuded surfaces so that drainage canals are created laterally, permitting the egress of uterine or cervical secretions. This procedure is usually restricted to those patients who are postmenopausal, are no longer interested in sexual activity, or have medical complications for which a rapid, noninvasive procedure is desirous.

The major disadvantages of this procedure are that (1) intercourse is limited and (2) the facility for evaluation of postmenupausal bleeding is compromised. Postmenopausal bleeding subsequent to this procedure poses difficulty in diagnosis and often predisposes the gynecologist to suboptimal procedures, as well as hazardous delay.

Dilatation and fractional curettage of the uterus may be precluded by vaginal obliteration and an abdominal hysterectomy may be required for diagnosis and treatment. In those cases with endometrial carcinoma, proper staging and review of histologic material was not carried out prior to surgery, compromising the patient's prognosis. In those cases where "breakdown" of the colpocleisis was successful and adequate diagnostic procedures were performed, the patient may be exposed to the risks of bladder and rectal injury.

At New York Medical College–Metropolitan Medical Center, we recently encountered one patient who presented with complaint of postmenopausal bleeding 3 years following a Le Fort procedure.

J. G., a 56-year-old woman, gravida 3, para 3-0-0-3, whose last menstrual period was in 1965, was admitted on September 22, 1974, for evaluation of postmenopausal bleeding. Her past surgical history included a Le Fort colpocleisis in 1971 for uterine prolapse and cystorectocele. She had been well until June, 1974, when she noted small amounts of intermittent vaginal bleeding. She was seen in the gynecology clinic on July 26, 1974. Pelvic examination revealed no abnormalities other than an obliterated vagina; the Pap smear was Class II, showing mild atrophic atypia.

The patient was admitted for a fractional dilatation and curettage. The cervix was approached by gradual dilatation of the right lateral drainage canal with lubricated Hegar dilators, as well as scalpel dissection of resistant fibrous bands. The previous surgery was not disrupted. The cervix was grasped, traction was applied, and fractional dilatation and curettage and cervical punch biopsies were performed. The pathology report revealed small amounts of moderately differentiated adenocarcinoma. Evaluation of metastatic sites of disease was negative. Our impression at that time was Stage Ia, Grade 2, endometrial carcinoma. Laparotomy revealed a 5 by 5 cm. left adnexal mass with papillary seeding on the Fallopian tubes, the uterus, and the right ovary. There was diffuse seeding of tumor over the serosal surfaces of the small intestine and omentum. The liver, spleen, and kidneys were normal. A total abdominal hysterectomy and bilateral salpingo-oophorectomy and omentectomy were performed. Extirpation of the cervix was not hindered by the prior Le Fort procedure. The bulk of the tumor was removed, leaving diffuse small metastatic

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Table I. Pelvic malignancy subsequent to a Le Fort procedure

Authors, year, and no. of cases of malignancy in series of Le Fort operations	*Diagnostic method*	*Histology*	*Further treatment*
Mazer and Israel[1] (1948) 1/43	TAH-BSO	Endometrial carcinoma	None
Falk and Kaufman[2] (1955) 1/100	TAH	Endometrial carcinoma	None
Hanson and Keetel[3] (1969) 1/288	D&C	Endometrial carcinoma	ICR* + TAH-BSO
Hanson and Keetel[3] (1969)	D&C, breakdown not mentioned	Endometrial carcinoma	ICR
Sudo, Goldberg, and Sall (1975) 1/74	D&C, no breakdown	Ovarian carcinoma	TAH-BSO + external teletherapy

*Intracavitary radium.

deposits. Surgical clips were placed in areas of larger metastatic concentrations. The pathology report revealed papillary adenocarcinoma of the left ovary. Postoperative cobalt-60 teletherapy was initiated on October 22, 1974 delivering 3,000 r to the whole abdomen in 4 weeks, given through AP-PA portals, blocking the kidneys posteriorly. This was followed by 2,000 r to the whole pelvis.

Pelvic malignancy following a Le Fort operation has been reported in only four instances, all of which have been endometrial in origin (Table I). There are no reported cases of carcinoma of the cervix.[3] This is the first reported case of ovarian malignancy following a Le Fort colpocleisis.

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Benign melanosis of the vagina and cervix

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Melanotic lesions of the vagina and cervix, benign or malignant, are rare. The majority of such lesions should be regarded as malignant until proved otherwise,[1] but there are occasional benign cases. This report illustrates a case of benign melanosis of the vagina and cervix in a 46-year-old woman which remained unchanged in a 13 year follow-up.

This 46-year-old black woman, gravida 4, para 2, abortus 2, was referred to this institute 13 years ago because of discoloration of the vaginal mucosa. She had had irregular and heavy menstruation for the last 6 years and had been under the care of a physician, who noted the discoloration recently. On examination at this institute, irregular patches of bluish discoloration were noted on the vagina and portio vaginalis. The vaginal lesion was on the anterior wall, beginning at the right anterior fornix, and involved the upper two thirds of the vaginal canal, measuring about 3 cm. in width. The area was neither raised nor firm and was normal on palpation. The cervical discoloration was noted at the 3 and 9 o'clock positions, each measuring about 0.7 cm.

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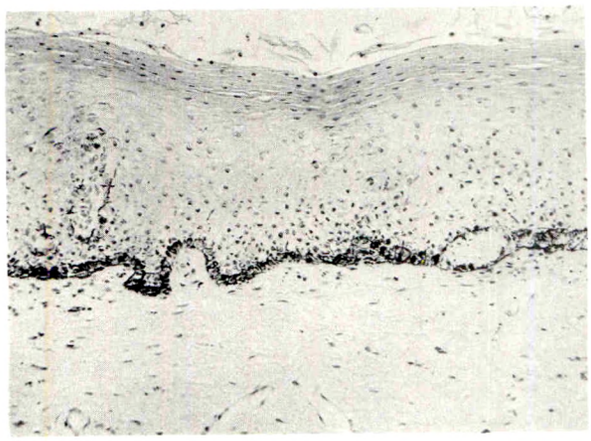


Fig. 1. Photomicrograph of the vaginal mucosa. (Section stained by Fontana stain for melanin; ×220.)

Biopsies of the lesions revealed pigmentation of the basal layer of the squamous epithelium with melanin pigment. Melanocytes with dendrites and melanin pigment were evident among pigmented epithelial cells (Fig. 1). She has been followed at the outpatient clinic and the lesions have not changed. Another biopsy, obtained recently, showed the same histologic feature.

Benign melanotic lesions of the vagina and/or cervix are rare and only few cases have been documented. Nigogosyan, de la Pava, and Pickren[2] studied the vaginal mucosa of 100 random autopsies and found a similar lesion to that of the present case in three cases. All three patients were elderly white women. In one case the pigmentation was extensive. This patient received radiation to the uterus for postmenopausal bleeding 9 months before death. In the other two the pigmented areas were small. In none of the cases were

the lesions recognized clinically. Norris and Taylor[1] described two benign melanotic lesions in the vagina; one resembled a lentigo and the other consisted of melanin-laden cells in the lamina propria. A case of melanin pigmentation of the basal layer of the cervical squamous epithelium was briefly mentioned by Ackerman and Rosai in their *Surgical Pathology* textbook. Other reported cases include pigmented nevus of the vagina and blue nevus of the cervix.

The present patient was referred to this Institute with suspicion of malignant melanoma of the vagina. Biopsy of the lesions, however, revealed melanin pigmentation of the basal layer of the squamous epithelium with scattered melanocytes. This histologic feature corresponds to melanosis of the mucosa found elsewhere. On physical examination the lesions were soft, not raised, and normal on palpation. No ulceration was present. This is in contrast with malignant melanoma of the vagina which is usually nodular, polypoid, or ulcerated, and bleeds easily when touched.[1]

The incidence of melanosis of the vagina and/or cervix is probably higher than one would expect from rarity of reported cases. This is suggested by the fact that Nigogosyan and associates[2] found three such lesions in 100 random autopsies. The lesion of this patient remained stable for 13 years and it is unlikely that it is a precursor of malignant melanoma. Preexisting, concurrent, or later developing melanosis is frequently noted in malignant melanoma of the oral cavity. This phenomenon, however, has not been described in malignant melanoma of the vagina or cervix. The origin of melanocytes in the vagina and cervix is still unsettled but most likely is the neural crest, as suggested by previous investigators.[1, 2]

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Chlordiazepoxide withdrawal in the neonate

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THE USE OF addictive drugs during pregnancy has risen sharply over the past decade. In the 1960's heroin was the major drug of abuse leading to the neonatal abstinence syndrome. In more recent years, the use of methadone, barbiturates, and tranquilizers, either alone or in combination, has been observed with increasing frequency. We have recently observed severe signs of withdrawl in twin infants of a mother who used chlordiazepoxide (Librium) throughout pregnancy.

A 33-year-old woman, para 1-0-0-1, who had been treated with chlordiazepoxide, 30 mg. per day for 5 years because of a moderately severe emotional disorder, had her dosage lowered to 20 mg. per day in the twelfth week of the present pregnancy. The use of other psychotropic drugs during pregnancy was denied. After a 36 week gestation, she delivered female monozygotic twins weighing 1,840 grams (Twin A) and 1,590 grams (Twin B), respectively.

The clinical course of both infants was uncomplicated during the first 3 weeks of life. On the twenty-first day, both infants were noted to be irritable, and coarse tremors were observed (more pronounced in Twin B than in Twin A). A diagnostic evaluation for infectious and metabolic disorders was negative, and a presumptive diagnosis of chlordiazepoxide withdrawal was made. Blood drawn from both infants at this time (umbilical cord serum was not available) was sent to Roche Laboratories, where analysis for chlordiazepoxide showed no trace of the drug. Twin B was treated with phenobarbital, 8 mg. per kilogram per day, given in three divided oral doses, while Twin A was swaddled and placed in a quiet environment.

Although there was a temporary improvement in the clinical condition of both infants, severe tremulousness and irritability were noted on day 26. Treatment with intramuscular diazepam, 0.5 mg. every 12 hours, was begun in both infants. Within 48 hours a marked improvement was noted. After 5 days, the dosage of diazepam was tapered and therapy discontinued after 9 days. The further clinical course of both infants remained uneventful.

Benzodiazepine compounds (chlordiazepoxide, diazepam, and oxazepam) are widely used tranquilizers which may cause physical dependence when used in large doses. Abstinence is associated with a clinical syndrome similar to that observed in withdrawal from barbiturates and meprobamate—depression, agitation, loss of appetite, insomnia, and, in some cases, actual convulsions.

Excretion of the drug, which is slow in adults, is markedly delayed in premature infants. Therefore, signs of withdrawal may be significantly delayed after transplacental exposure to the drug has ceased. This is similar to neonatal withdrawal from methadone[1] and long-acting barbiturates,[2] where initial signs of abstinence may be delayed for 2 to 4 weeks after birth.

Although initial signs of neonatal withdrawal from diazepam have been observed as late as 10 days of age, we are not aware of any reports of chlordiazepoxide withdrawal in the neonate.

While the diagnosis in our cases is presumptive

rather than proved, we would nonetheless caution physicians caring for infants of mothers who received benzodiazepine compounds during pregnancy to be alert for a delayed withdrawal syndrome, especially after the infant has been discharged from the newborn nursery. Clinical observations should be correlated with concentrations of the drug in umbilical cord serum and blood of the infant.

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CORRESPONDENCE

Progesterone-releasing intrauterine contraceptive device

To the Editors:

I read with great interest the report of Brenner and associates (AM. J. OBSTET. GYNECOL. 121: 704, 1975) on a progesterone-releasing intrauterine contraceptive device.

We tested the same devices by inserting in a randomized fashion 99 T-devices releasing 65 μg of progesterone daily and 99 "placebo" devices obtained under the auspices of the World Health Organization. Since both trials were conducted by similar methods, readers may be interested in our results, with differences in the population and in the habits and attitudes of the patients, which may affect the over-all acceptability pattern, kept in mind. Such local factors may account for the fact that our discontinuation rate was so much lower than that of Brenner and associates.

We inserted devices from January to May, 1973, during menstruation in 198 women, all of whom had regular menses and normal pelvic findings. The patients were followed systematically, and the effectiveness of the devices was computed by the "life-table method" of Tietze.

Our results are shown in Table I.

After insertion, irregular bleeding occurred in 130 patients up to the second follow-up visit (third month). At the one-month checkup, 102 women reported irregular bleeding, while a total of 63 women reported bleeding during the second and/or third month. With exclusion of patients requesting removal, bleeding stopped spontaneously or after treatment with uterotonic agents after one to three weeks. After the third month, only 13 instances of spotting and one instance of bleeding occurred.

It should be mentioned that the devices were not immediately removed at the first report of bleeding, Removal was performed only in women who continued bleeding at least three weeks following unsuccessful treatment with uterotonic agents. Removal had to be performed in only seven of 130 patients, six of whom had irregular bleeding during the first three months. With this management, there was a very marked reduction in removal for bleeding as compared with the results reported by Brenner and his co-workers.

The one-year cumulative rate for wearing the devices per 100 women was 83.84; for the progesterone type it was 85.86, and for the placebo device it was 81.82 (with 9 pregnancies). With exclusion of removals for nonmedical reasons, the cumulative wearing rates became: total, 86.37; the progesterone device, 86.87; and the placebo device, 85.86.

The incidence of removal of the device for bleeding may be minimized if the woman is informed that some bleeding is common in the first three months and that it occurs relatively infrequently after the fourth month. A patient, positive, communicative approach on the part of the physician encourages the woman to leave the device in place during the crucial first three months. It may be worth noting that, in the Brenner series and in our series, the incidence of removal for bleeding was greater in women with the progesterone device than in women with the placebo device, the totals being 24 and 12, respectively. Also, the progesterone devices in our series tended to be associated with alteration of the menstrual cycle. We are studying the clinical significance of these phenomena.

Intensive evaluation of 10 of the patients with the progesterone device and 10 of the patients with the placebo device failed to reveal any difference in the serum progesterone levels or in pregnanediol and estriol excretion from the normal values, proving that the progesterone release acts only locally. Also the

Table I. Number of events causing termination for each device

Event	*Device*	
	Medicated	*Inert*
No. of women	99	99
No. of woman months	1,324	1,270
Pertinent discontinuations		
Accidental pregnancy	0	9
Expulsion	4	2
Bleeding	4	1
Bleeding plus inflammation	2	0
Inflammation	3	2
Pain	0	2
Total	13	16
Other		
Planning pregnancy	0	2
Other personal	1	0
Total	1	2
Total discontinuations	14	18
Lost to follow-up	none	none

This investigation received financial support from the World Health Organization.

basal temperatures were biphasic. At endometrial biopsy, predecidual changes were found.

To eliminate the relatively high occurrence of initial irregular bleeding which suggests removal, determining the optimal content and rate of progesterone release should provide a combination method having the advantage of both hormonal and local (mechanical and hormonal) acting methods of fertility control.

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Sterilization guidelines

To the Editors:

Recent urging of the need for sterilization guidelines, to determine whether requests will or will not be complied with, has been attacked as "male chauvinist control" over the life and reproduction of women and as an infringement of civil rights.

Since it is clear that sterilization as a permanent irreversible removal of the childbearing *option* is to be viewed as a last resort, it should be equally clear that evaluation of motivations and alternatives is necessary and that no request for any elective operation can be mandatorily complied with regardless of data elicited or clinical judgement. A basic medical-surgical principle states that *no* elective irreversible extirpation of structure or function should ever be carried out unless all reversible alternatives have been *genuinely* exhausted. An equally solid medical-surgical principle states that the *least* chemical-physical ministration which achieves a desired goal is preferred to any more extensive ministration. The burden of proof is on any practitioner who violates these principles. We know that an extremely important component of body image, self-image, or ego identity is intact anatomic structure and intact physiologic options. The permanent loss of any human functional option, of which *potential* generativity is a central example, cannot fail to reduce the human sense of self in the loser.

In view of the above, in a person requesting sterilization of any age or parity, with or without past exposure to reversible contraceptive counseling and methods, regardless of the degree of self-hatred, self-contempt, rejection of gender, and self-mutilating motivation elicited on competent interview, and regardless of the degree of high acute stress which might be contaminating the decision (as in current unwanted pregnancy and abortion or imminent emergency cesarean section), to insist that regardless of all such clinical data the request must be complied with *at that time,* in defense of the civil libertarian, democratic freedom of the patient to be sterilized on request, indeed represents the height of professional abdication and irresponsibility and should be opposed by all conscientious clinicians.

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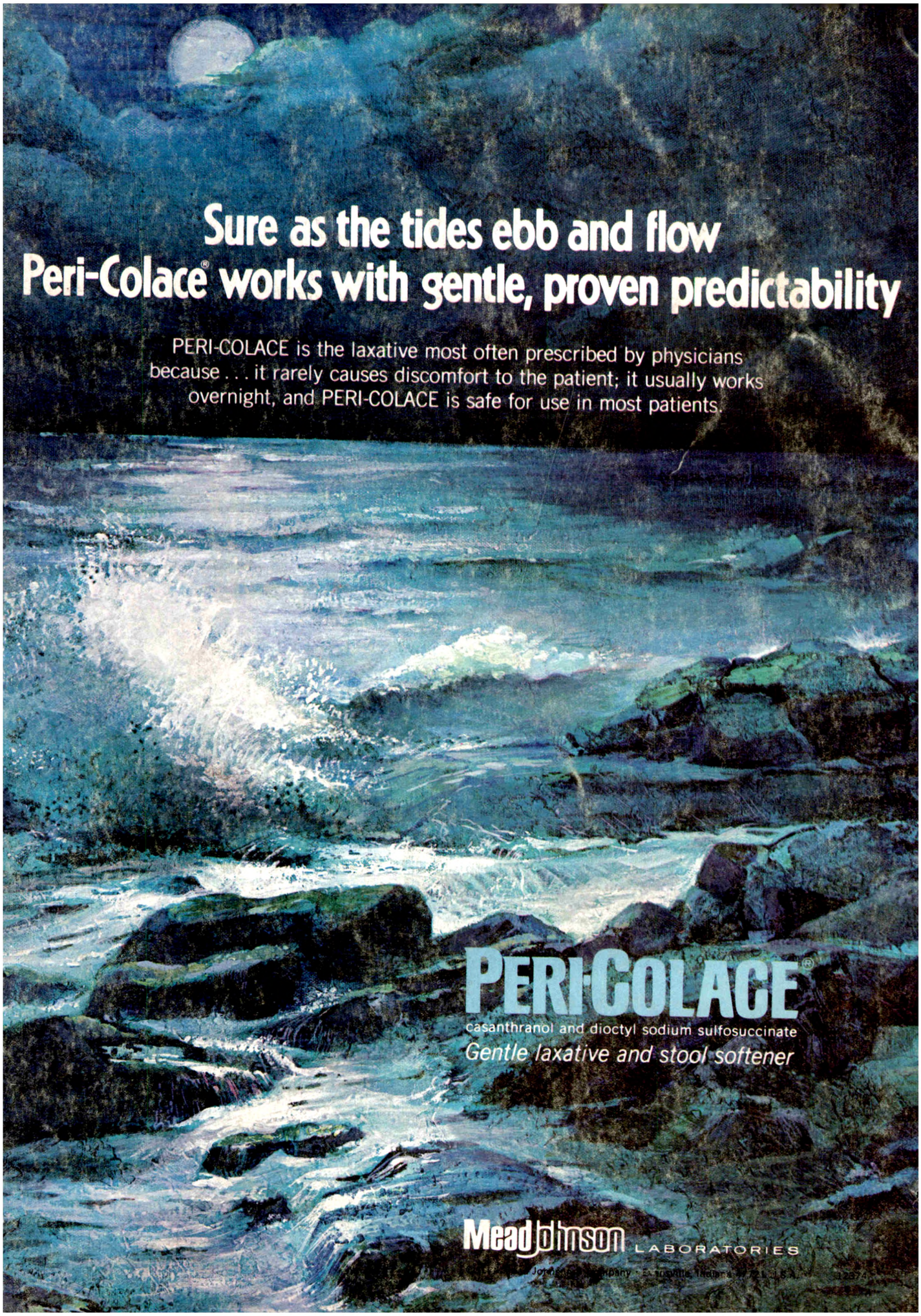
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NORINYL® 1+50
tablets (norethindrone 1 mg with mestranol 0.05 mg)

Special Note

Oral contraceptives have been marketed in the United States since 1960. Reported pregnancy rates vary from product to product. The effectiveness of the sequential products appears to be somewhat lower than that of the combination products. Both types provide almost completely effective contraception.

An increased risk of thromboembolic disease associated with the use of hormonal contraceptives has now been shown in studies conducted in both Great Britain and the United States. Other risks, such as those of elevated blood pressure, liver disease and reduced tolerance to carbohydrates, have not been quantitated with precision.

Long term administration of both natural and synthetic estrogens in subprimate animal species in multiples of the human dose increases the frequency for some animal carcinomas. These data cannot be transposed directly to man. The possible carcinogenicity due to the estrogens can neither be affirmed nor refuted at this time. Close clinical surveillance of all women taking oral contraceptives must be continued.

Indications

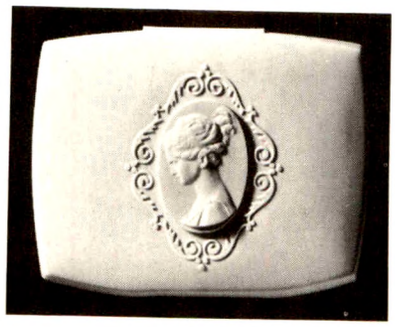
NORINYL 1+50 and NORINYL 1+80 are indicated for oral contraception.

Contraindications

1. Thrombophlebitis, thromboembolic disorders, cerebral apoplexy, or a past history of these conditions.
2. Markedly impaired liver function.
3. Known or suspected carcinoma of the breast.
4. Known or suspected estrogen dependent neoplasia.
5. Undiagnosed abnormal genital bleeding.
6. Known or suspected pregnancy.

Warnings

1. The physician should be alert to the earliest manifestations of thrombotic disorders (thrombophlebitis, cerebrovascular disorders, pulmonary embolism, and retinal thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. Retrospective studies of morbidity and mortality in Great Britain and studies of morbidity in the United States have shown a statistically significant association between thrombophlebitis, pulmonary embolism, and cerebral thrombosis and embolism and use of oral contraceptives. There have been three principal studies in Britain[1,2,3] leading to this conclusion, and one[4] in this country. The estimate of the relative risk of thromboembolism in the study by Vessey and Doll[3] was about sevenfold, while Sartwell and associates in the United States found a relative risk of 4.4, meaning that the users are several times as likely to undergo thromboembolic disease without evident cause as non-users. The American study also indicated that the risk did not persist after discontinuation of administration, and that it was not enhanced by long continued administration. The American study was not designed to evaluate a difference between products. However, the study suggested that there might be an increased risk of thromboembolic disease in users of sequential products. This risk cannot be quantitated, and further studies to confirm this finding are desirable.
2. Discontinue medication pending examination if there is sudden partial or complete loss of vision, or if there is a sudden onset of proptosis, diplopia or migraine. If examination reveals papilledema or retinal vascular lesions, medication should be withdrawn.
3. Since the safety of NORINYL 1+50 and NORINYL 1+80 in pregnancy has not been demonstrated, it is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule the possibility of pregnancy should be considered at the time of the first missed period.
4. A small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The long range effect to the nursing infant cannot be determined at this time.
5. Hepatic cell adenomas have been reported in women on oral contraceptives. The adenoma may present as an abdominal mass or with the signs and symptoms of an acute abdomen. A bleeding hepatic cell adenoma should be considered if the patient has abdominal pain or evidence of intra-abdominal bleeding.



Norethindrone/mestranol 1+50 combination. From the company that developed norethindrone.

Precautions

1. The pretreatment and periodic physical examinations should include special reference to liver, breasts and pelvic organs, including Papanicolaou smear since estrogens have been known to produce tumors, some of them malignant, in five species of subprimate animals.
2. Endocrine and possibly liver function tests may be affected by treatment with NORINYL 1+50 and NORINYL 1+80. Therefore, if such tests are abnormal in a patient taking NORINYL 1+50 and NORINYL 1+80, it is recommended that they be repeated after the drug has been withdrawn for 2 months.
3. Under the influence of estrogen-progestogen preparations, pre-existing uterine fibromyomata may increase in size.
4. Because these agents may cause some degree of fluid retention, conditions which might be influenced by this factor, such as epilepsy, migraine, asthma, cardiac or renal dysfunction, require careful observation.
5. In breakthrough bleeding, and in all cases of irregular bleeding per vaginam, nonfunctional causes should be borne in mind. In undiagnosed bleeding per vaginam, adequate diagnostic measures are indicated.
6. Patients with a history of psychic depression should be carefully observed and the drug discontinued if the depression recurs to a serious degree.
7. Any possible influence of prolonged NORINYL 1+50 and NORINYL 1+80 therapy on pituitary, ovarian, adrenal, hepatic or uterine function awaits further study.
8. A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. The mechanism of this decrease is obscure. For this reason, diabetic patients should be carefully observed while receiving NORINYL 1+50 and NORINYL 1+80 therapy.
9. The age of the patient constitutes no absolute limiting factor, although treatment with NORINYL 1+50 and NORINYL 1+80 may mask the onset of the climacteric.
10. The pathologist should be advised of NORINYL 1+50 and NORINYL 1+80 therapy when relevant specimens are submitted.
11. Susceptible women may experience an increase in blood pressure following administration of contraceptive steroids.

Adverse Reactions Observed in Patients Receiving Oral Contraceptives

A statistically significant association has been demonstrated between use of oral contraceptives and the following serious adverse reactions: thrombophlebitis, pulmonary embolism, cerebral thrombosis.

Although available evidence is suggestive of an association, such a relationship has been neither confirmed nor refuted for the following serious adverse reactions: neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions are known to occur in patients receiving oral contraceptives: nausea, vomiting, gastrointestinal symptoms (such as abdominal cramps and bloating), breakthrough bleeding, spotting, change in menstrual flow, amenorrhea during and after treatment, edema, chloasma or melasma, breast changes: tenderness, enlargement and secretion, change in weight (increase or decrease), changes in cervical erosion and cervical secretions, suppression of lactation when given immediately post-partum, cholestatic jaundice, migraine, rash (allergic), rise in blood pressure in susceptible individuals, mental depression.

Although the following adverse reactions have been reported in users of oral contraceptives, an association has been neither confirmed nor refuted: anovulation post treatment, premenstrual-like syndrome, changes in libido, changes in appetite, cystitis-like syndrome, hirsutism, loss of scalp hair, erythema multiforme, headache, nervousness, dizziness, fatigue, backache, erythema nodosum, hemorrhagic eruption, itching.

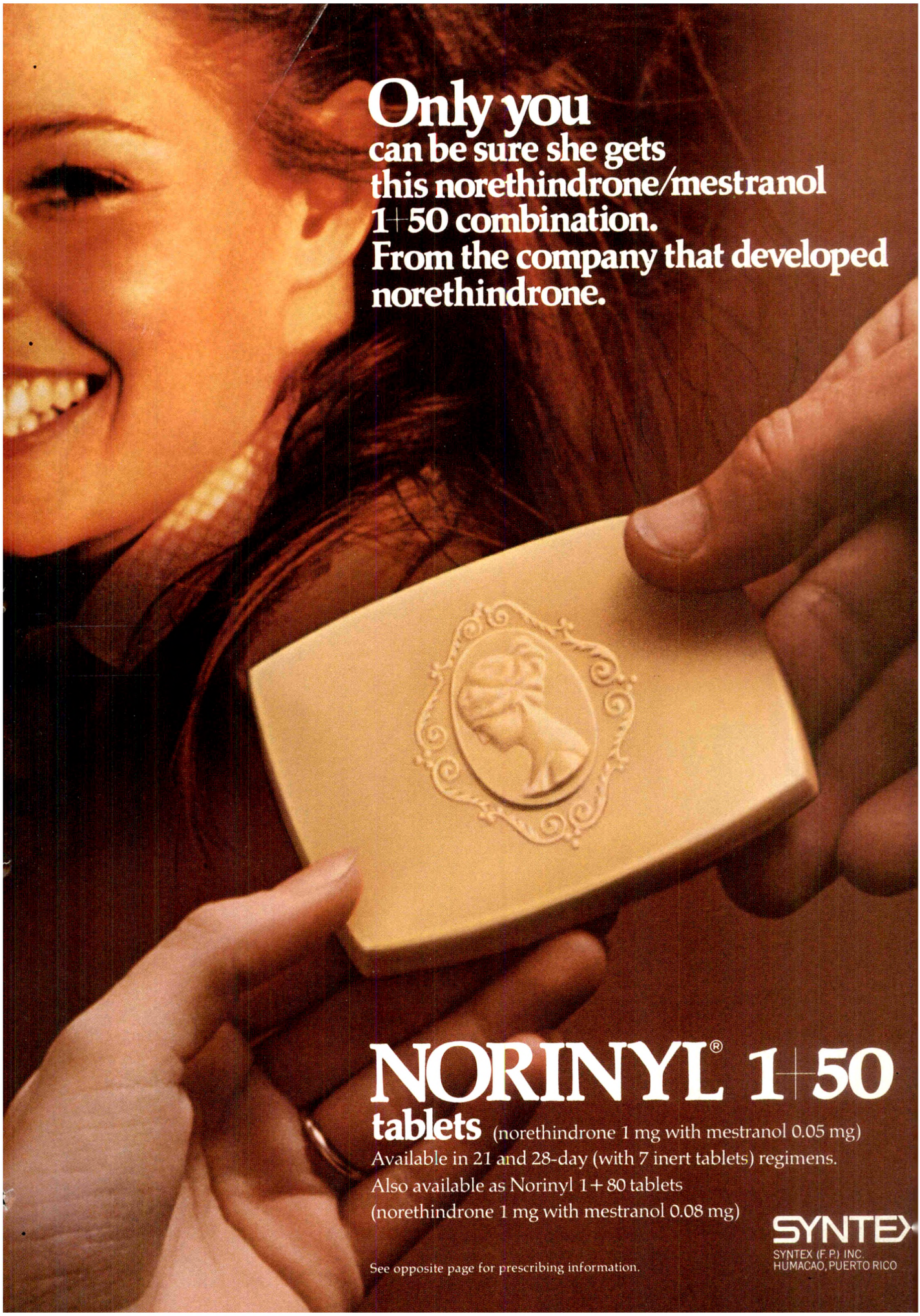
The following laboratory results may be altered by the use of oral contraceptives: hepatic function: increased sulfobromophthalein retention and other tests, coagulation tests: increase in prothrombin factors VII, VIII, IX, and X, thyroid function: increase in PBI, and butanol extractable protein bound iodine and decrease in T^3 uptake values, metyrapone test, pregnanediol determination.

[1]Royal College of General Practitioners: Oral Contraception and Thromboembolic Disease. J. Coll. Gen. Pract., 13:267-279, 1967.
[2]Inman, W.H.W. and Vessey, M.P. Investigation of Deaths from Pulmonary Coronary and Cerebral Thrombosis and Embolism in Women of Child-Bearing Age, Brit Med J, 2:193-199, 1968.
[3]Vessey, M.P. and Doll, R. Investigation of Relation between Use of Oral Contraceptives and Thromboembolic Disease. A Further Report. Brit Med J, 2:651-657, 1969.
[4]Sartwell, P.E., Masi, A.T., Arthes, F.G., Greene, G.R. and Smith, H.E., Thromboembolism and Oral Contraceptives: An Epidemiological Case-Control Study. Am. J.Epidem. 90:365-380, (Nov.) 1969.

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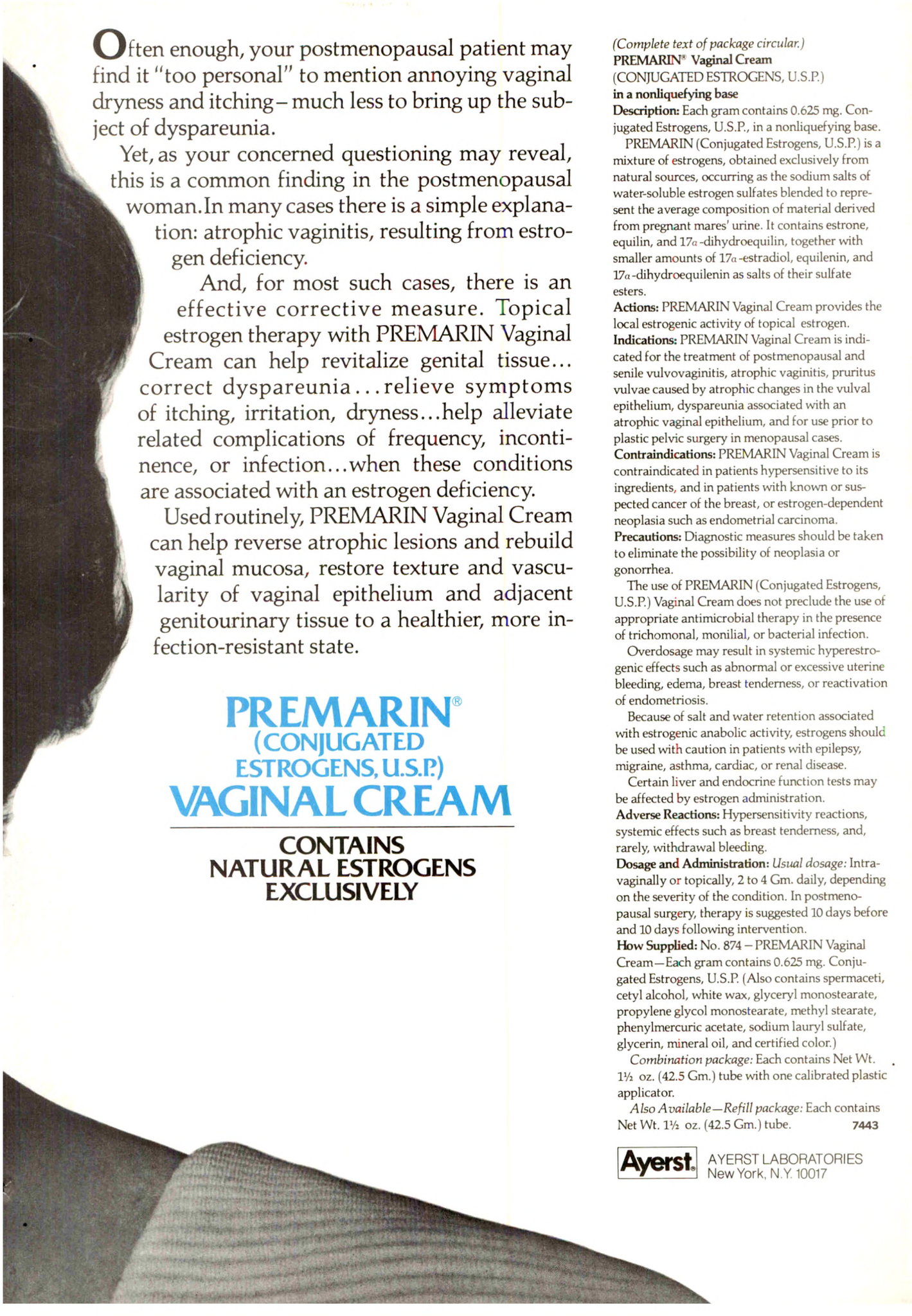
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in a nonliquefying base

Description: Each gram contains 0.625 mg. Conjugated Estrogens, U.S.P., in a nonliquefying base.

PREMARIN (Conjugated Estrogens, U.S.P.) is a mixture of estrogens, obtained exclusively from natural sources, occurring as the sodium salts of water-soluble estrogen sulfates blended to represent the average composition of material derived from pregnant mares' urine. It contains estrone, equilin, and 17α-dihydroequilin, together with smaller amounts of 17α-estradiol, equilenin, and 17α-dihydroequilenin as salts of their sulfate esters.

Actions: PREMARIN Vaginal Cream provides the local estrogenic activity of topical estrogen.

Indications: PREMARIN Vaginal Cream is indicated for the treatment of postmenopausal and senile vulvovaginitis, atrophic vaginitis, pruritus vulvae caused by atrophic changes in the vulval epithelium, dyspareunia associated with an atrophic vaginal epithelium, and for use prior to plastic pelvic surgery in menopausal cases.

Contraindications: PREMARIN Vaginal Cream is contraindicated in patients hypersensitive to its ingredients, and in patients with known or suspected cancer of the breast, or estrogen-dependent neoplasia such as endometrial carcinoma.

Precautions: Diagnostic measures should be taken to eliminate the possibility of neoplasia or gonorrhea.

The use of PREMARIN (Conjugated Estrogens, U.S.P.) Vaginal Cream does not preclude the use of appropriate antimicrobial therapy in the presence of trichomonal, monilial, or bacterial infection.

Overdosage may result in systemic hyperestrogenic effects such as abnormal or excessive uterine bleeding, edema, breast tenderness, or reactivation of endometriosis.

Because of salt and water retention associated with estrogenic anabolic activity, estrogens should be used with caution in patients with epilepsy, migraine, asthma, cardiac, or renal disease.

Certain liver and endocrine function tests may be affected by estrogen administration.

Adverse Reactions: Hypersensitivity reactions, systemic effects such as breast tenderness, and, rarely, withdrawal bleeding.

Dosage and Administration: *Usual dosage:* Intravaginally or topically, 2 to 4 Gm. daily, depending on the severity of the condition. In postmenopausal surgery, therapy is suggested 10 days before and 10 days following intervention.

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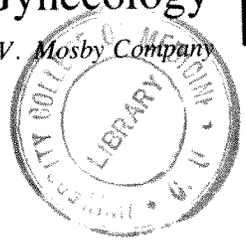
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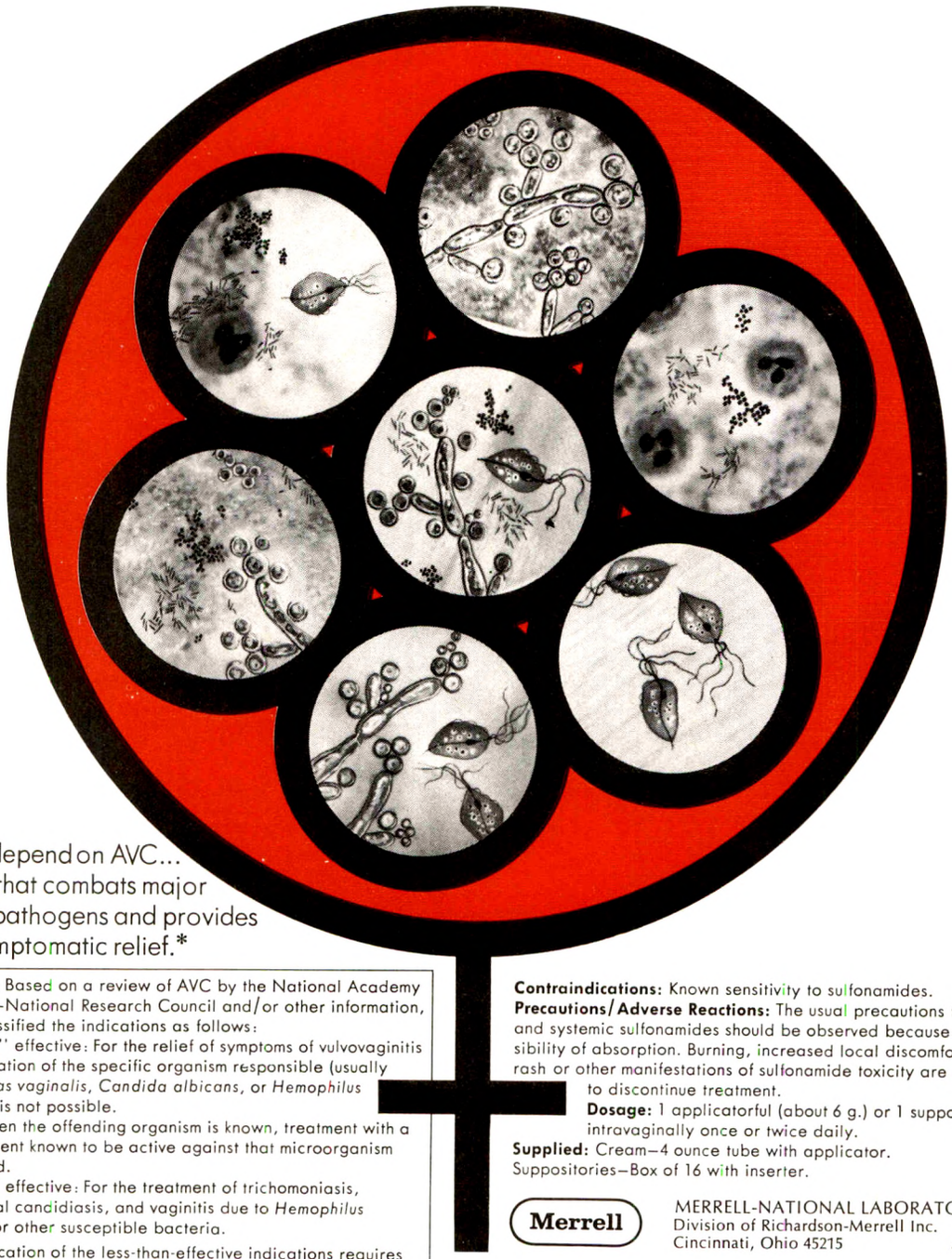
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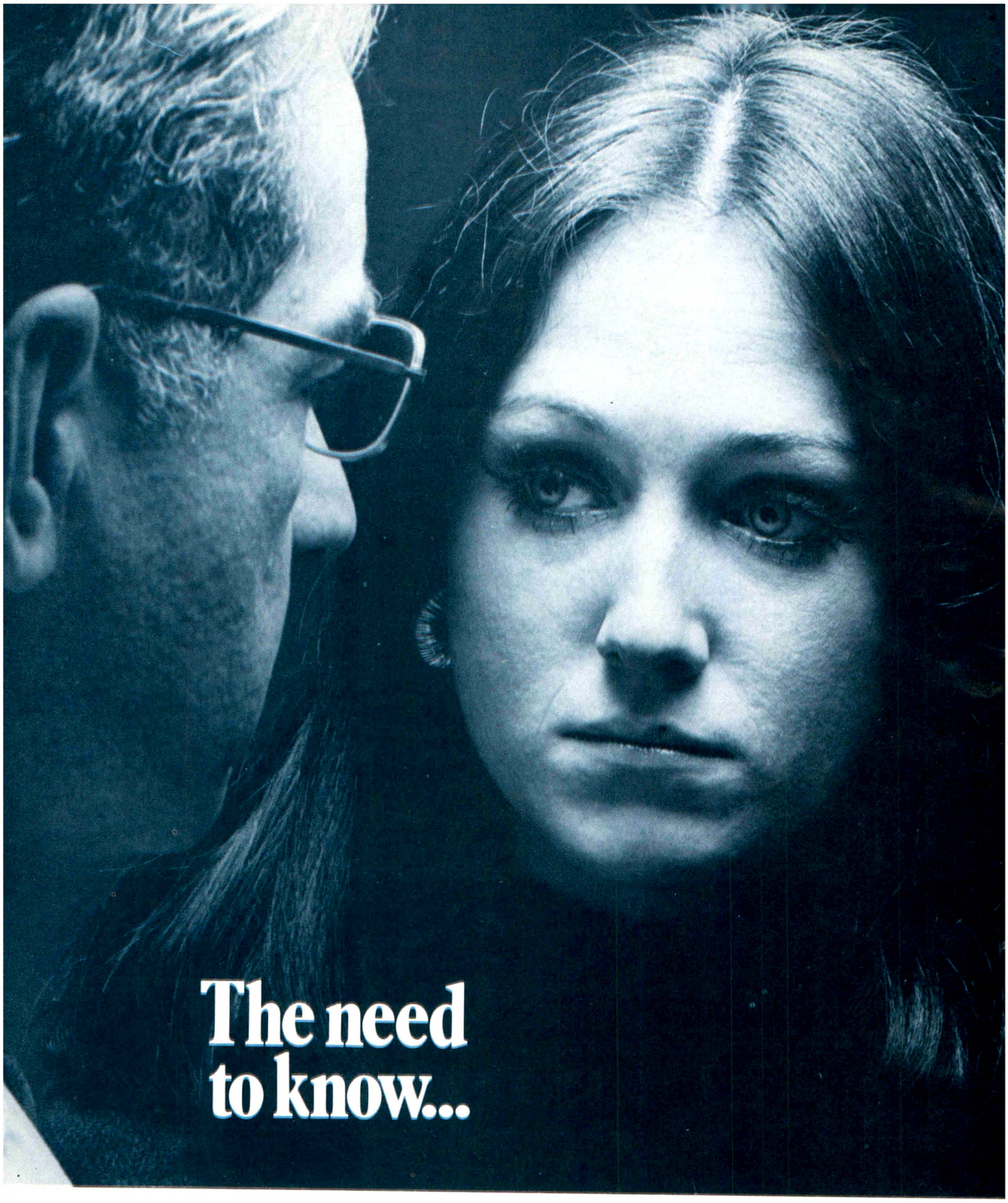
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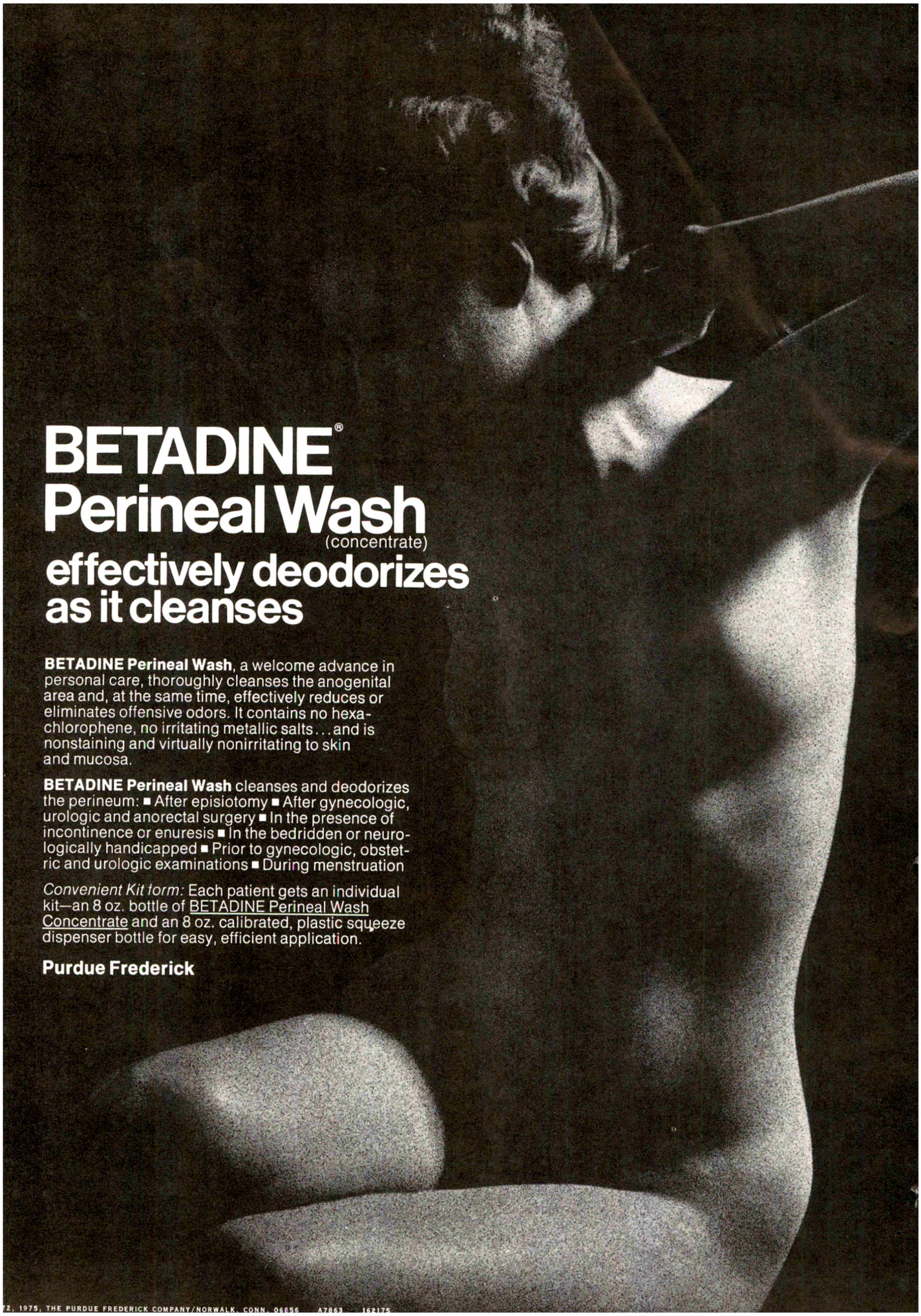
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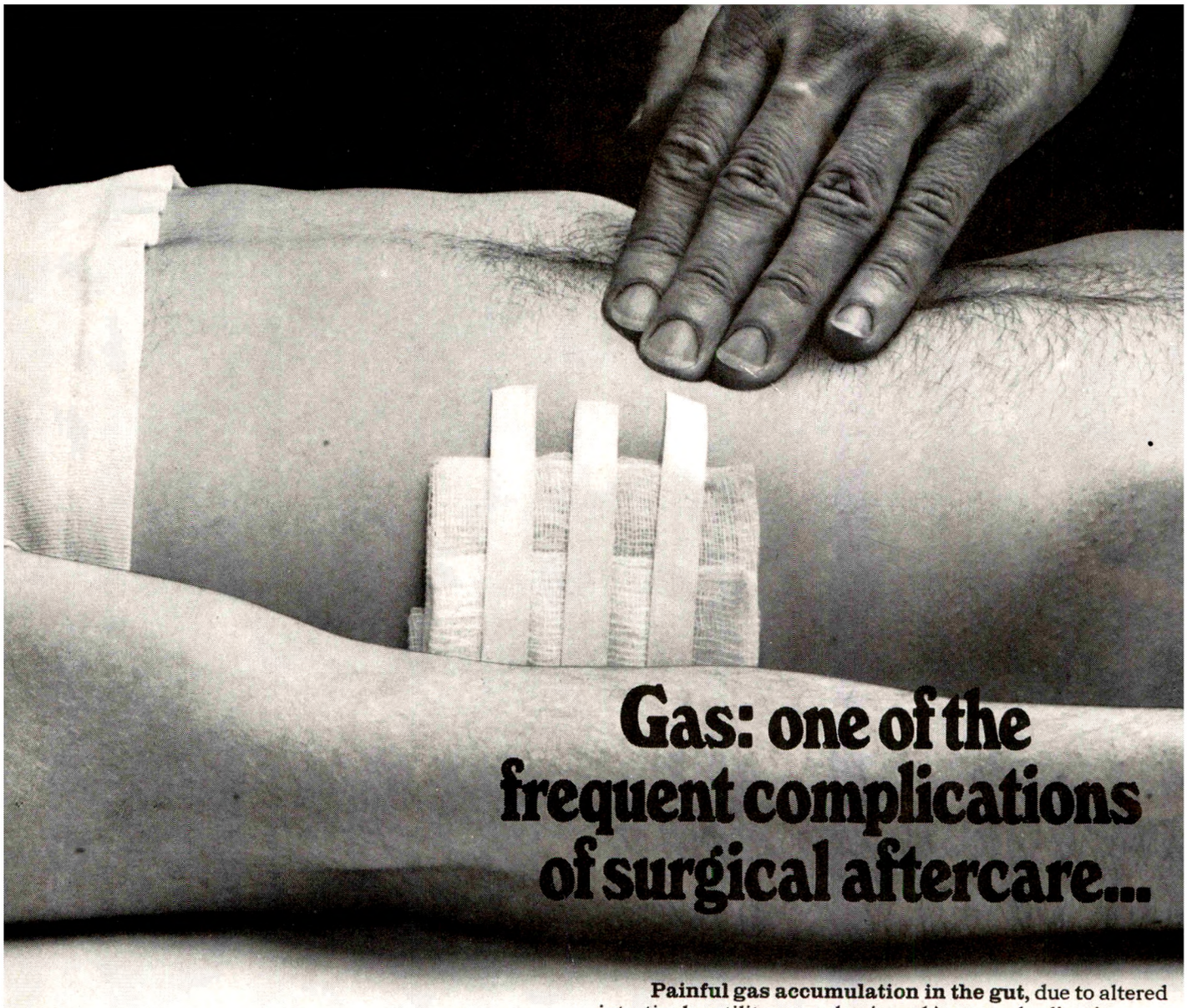
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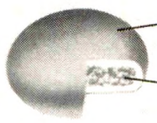
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1. Gibstein, A., *et al.*: Prevention of postoperative abdominal distention and discomfort with simethicone, *Obstet. Gynecol. 38*:386, Sept. 1971.
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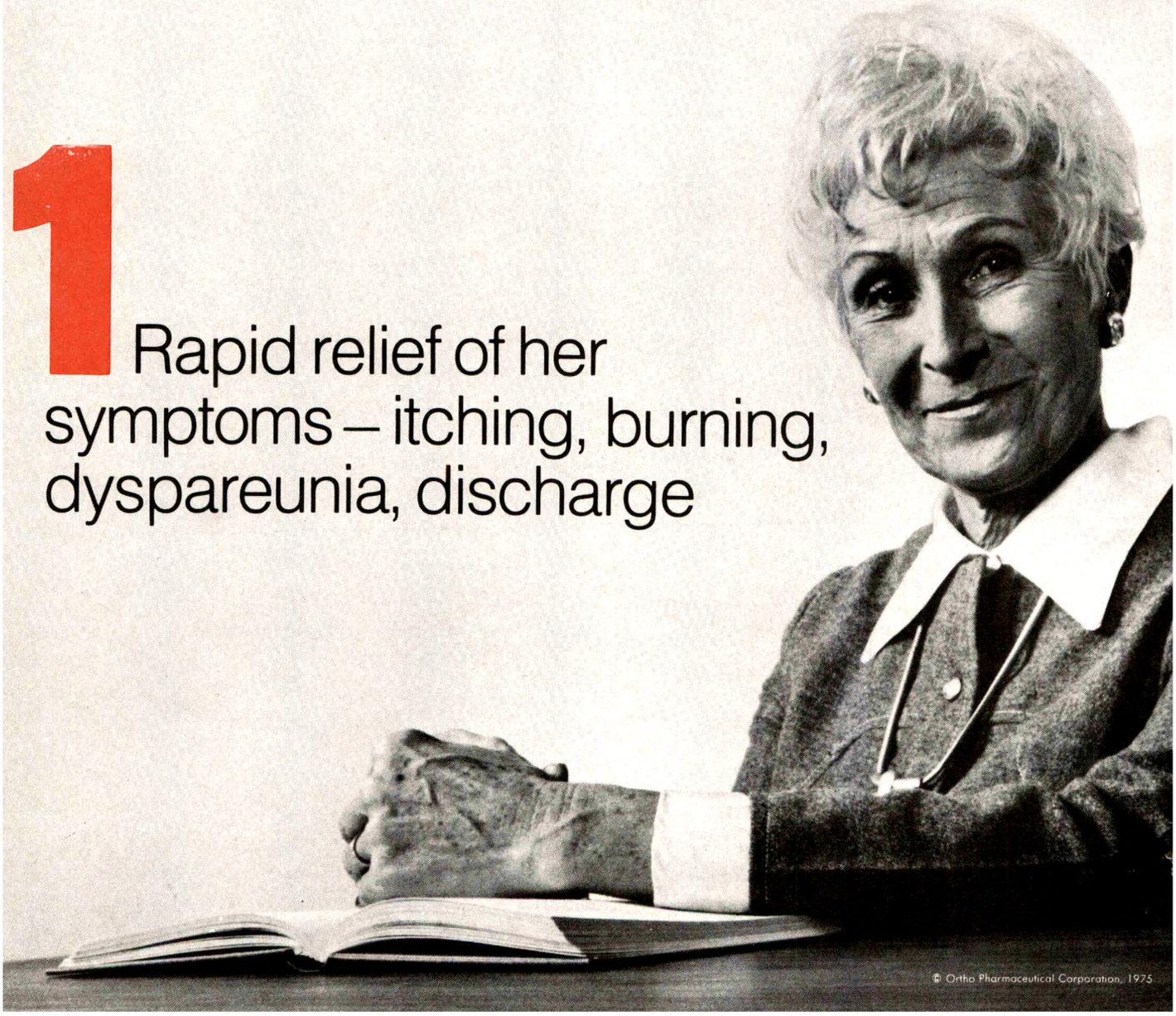
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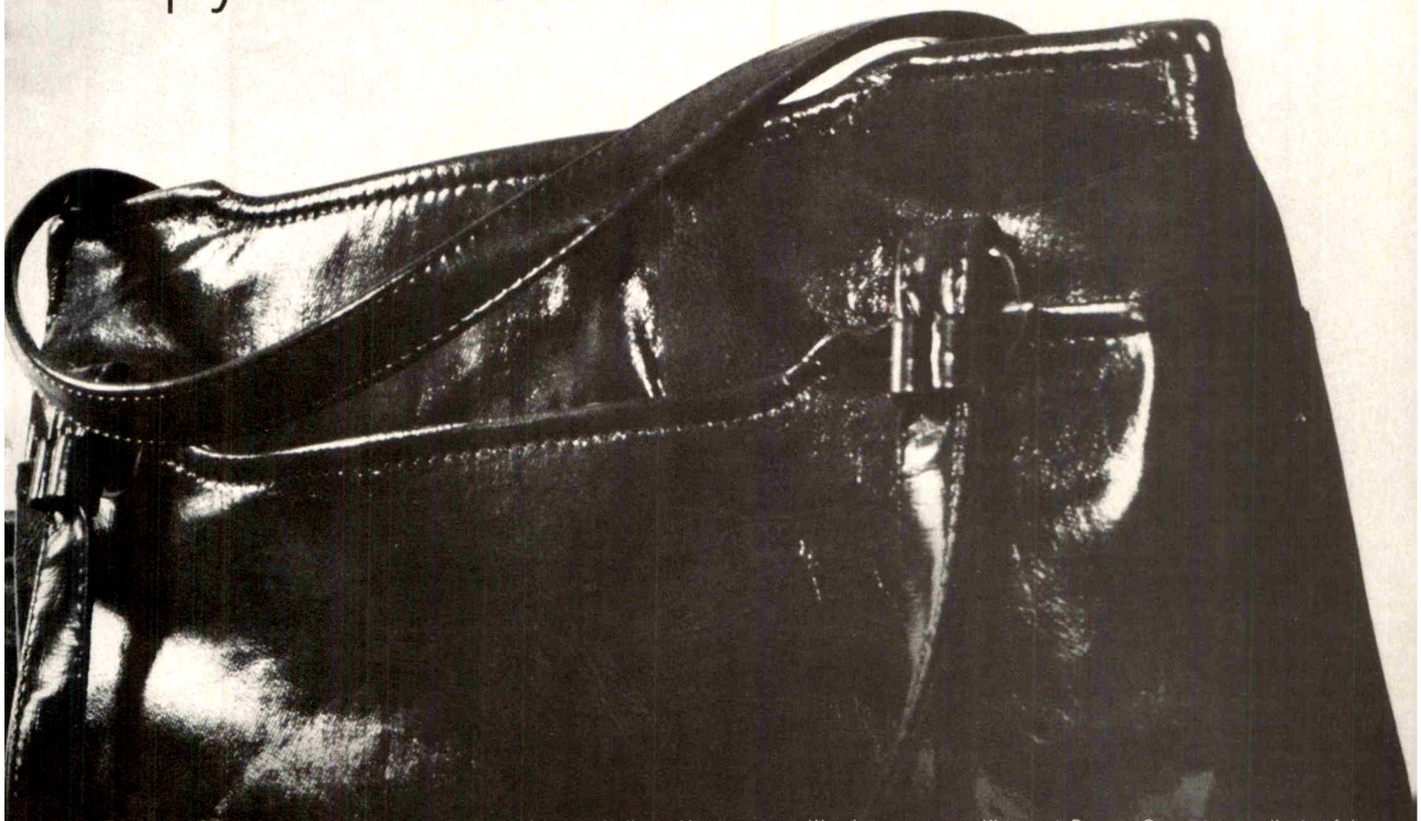
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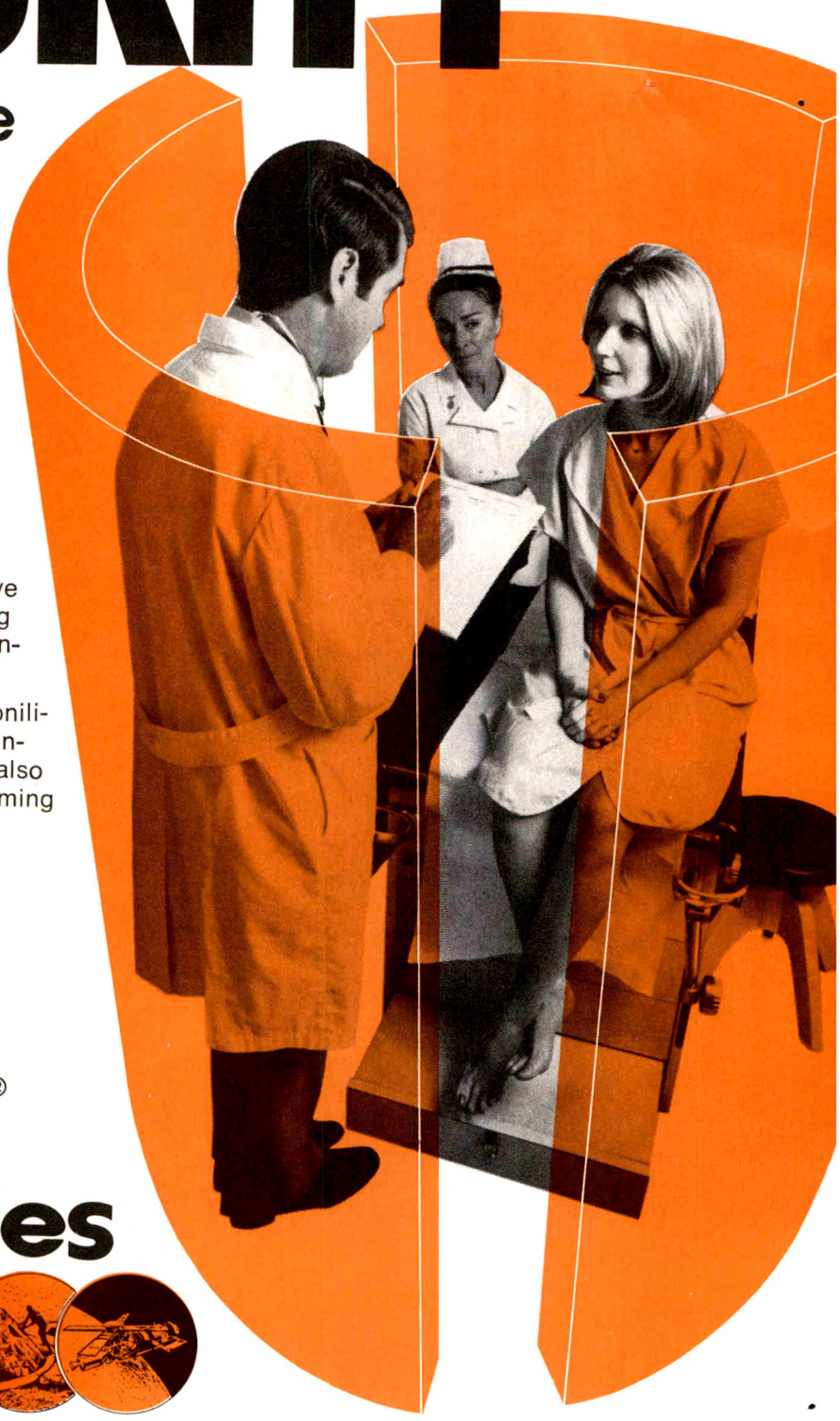
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Incidence of Breakthrough Bleeding in Premarketing Clinical Trials

Cycle	1	3	6	12	Total Cycles 11,085
Breakthrough Bleeding	9.0%	3.5%	2.7%	2.6%	3.9%

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*Serious as well as minor adverse reactions have been reported following the use of all oral contraceptives. The physician should remain alert to their earliest manifestations and in the event of serious adverse reactions should avoid any oral contraceptives. *Special note, contraindications, warnings, precautions and adverse reactions* in the accompanying IN BRIEF should be carefully considered.

**OVRAL® each tablet contains 0.5 mg. norgestrel with 0.05 mg. ethinyl estradiol, Wyeth.

Important information on next page.

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Low dose oral contraceptive.

IN BRIEF:

SPECIAL NOTE: Oral contraceptives have been marketed in the United States since 1960. Reported pregnancy rates vary from product to product. The effectiveness of the sequential products appears to be somewhat lower than that of the combination products. Both types provide almost completely effective contraception.

An increased risk of thrombotic, thromboembolic and cerebral-vascular disease associated with the use of hormonal contraceptives has been shown in studies conducted in both Great Britain and the United States. Other risks, such as those of elevated blood pressure and reduced tolerance to carbohydrates, have been reported, and appropriate tests should be conducted to monitor these during oral-contraceptive therapy. Liver disease has also been reported, and the physician should be alert to its earliest manifestations.

Long-term administration of both natural and synthetic estrogens in subprimate animal species in multiples of the human dose increases the frequency for some animal carcinomas. These data cannot be transposed directly to man. The possible carcinogenicity due to the estrogens can neither be affirmed nor refuted at this time. Close clinical surveillance of all women taking oral contraceptives must be continued.

INDICATIONS: For oral contraception.

CONTRAINDICATIONS: 1. Thrombophlebitis, thromboembolic disorders, cerebral-vascular disease, or a past history of these conditions; 2. Markedly impaired liver function; 3. Known or suspected carcinoma of the breast; 4. Known or suspected estrogen-dependent neoplasia; 5. Undiagnosed abnormal genital bleeding; 6. Known or suspected pregnancy.

WARNINGS: 1. The physician should be alert to the earliest manifestations of thrombotic and thromboembolic disorders, thrombophlebitis, cerebrovascular disorders including hemorrhage, pulmonary embolism and retinal thrombosis. Should any of these occur or be suspected, the drug should be discontinued immediately.

Retrospective studies of morbidity and mortality in Great Britain and studies of morbidity in the United States have shown a statistically significant association between thrombophlebitis, pulmonary embolism and cerebral-vascular disease, occlusive or hemorrhagic, and the use of oral contraceptives. There have been three principal studies in Great Britain[1-3] leading to these conclusions and three[4-7] in this country. The estimate of the relative risk of thromboembolism in the study by Vessey and Doll[3] was about sevenfold, while the United States studies found relative risks of 4.4 to 11, meaning that the users are several times as likely to undergo thromboembolic disease without evident cause as non-users.

In May, 1974, the Royal College of General Practitioners in England[8] issued an interim report of its continuing large-scale prospective study comparing a user group to a non-user group. This study in its interim analysis states: "A statistically significant higher rate of reporting of cerebrovascular accidents in Takers is evident, but the numbers are too small to justify an estimation of the degree of risk." The study also reported a higher incidence of superficial and deep vein thrombosis in users as compared to non-users.

The Sartwell study[4] indicated that the risk did not persist after discontinuation of administration. Both the Sartwell and the Royal College study indicated that the degree of risk was not associated with duration of treatment.

In a collaborative American study[5,6] of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the relative risk of thrombotic stroke was 4.1 to 9.5 times greater in users than in non-users. A comparable estimate for hemorrhagic stroke was 2.0.

None of the American studies was designed to evaluate a difference between products. However, the Sartwell study[4] suggested that there might be an increased risk of thromboembolic disease in users of sequential products.

Other retrospective studies[9,10] have reported an increased risk of post-surgery thromboembolic complications in oral-contraceptive users. It has been recommended that therapy be discontinued at least one month prior to elective surgery.

2. Discontinue oral-contraceptive medication if there is: gradual or sudden, partial or complete loss of vision; proptosis or diplopia; onset or aggravation of migraine or development of headache of a new pattern which is recurrent, persistent or severe; papilledema; or any evidence of retinal-vascular lesions. 3. Fetal abnormalities have been reported to occur in the offspring of women who have taken progestogens and/or estrogens during pregnancy.[11,12] The safety of LO/OVRAL® in pregnancy has not been demonstrated. Pregnancy should be ruled out before initiating or continuing the contraceptive regimen. Pregnancy should always be considered if withdrawal bleeding does not occur. 4. A small fraction of the hormonal agent in oral contraceptives has been identified in the milk of mothers receiving these drugs. The long-range effect to the nursing infant cannot be determined at this time. 5. Hepatic lesions (adenomas, hepatomas, hamartomas, regenerating nodules, etc.), occasionally fatal, have been reported in women on oral contraceptives. Such lesions may present as an abdominal mass or with the signs and symptoms of an acute abdomen. These lesions should be considered if the patient has abdominal pain or evidence of intra-abdominal bleeding. Such lesions have been reported in short-term as well as long-term users of oral contraceptives.

PRECAUTIONS: 1. A thorough history and physical examination should be performed before prescribing oral contraceptives and periodically during their administration and should include special reference to breasts and pelvic organs including Papanicolaou smears, since estrogens have been known to produce tumors, some of them malignant, in five species of subprimate animals. 2. Endocrine and possibly liver-function tests may be affected by treatment with LO/OVRAL® Therefore, if such tests are abnormal in a patient taking LO/OVRAL® it is recommended that they be repeated after the drug has been withdrawn for two months. 3. Under the influence of estrogen-progestogen preparations, pre-existing uterine fibromyomata may increase in size. 4. Because these agents may cause some degree of fluid retention, conditions which might be influenced by this factor, such as epilepsy, migraine, asthma, cardiac or renal dysfunction, require careful observation. 5. In breakthrough bleeding, and in all cases of irregular bleeding per vaginam, nonfunctional causes should be borne in mind. In undiagnosed bleeding per vaginam, adequate diagnostic measures are indicated. 6. Patients with a history of psychic depression should be carefully observed and the drug discontinued if the depression recurs to a serious degree. 7. Any possible influence of prolonged LO/OVRAL® therapy on pituitary, ovarian, adrenal, hepatic or uterine function awaits further study. 8. A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. The mechanism of this decrease is obscure. For this reason, diabetic patients should be carefully observed while receiving LO/OVRAL® therapy. 9. The age of the patient constitutes no absolute limiting factor, although treatment with LO/OVRAL® may mask the onset of the climacteric. 10. The pathologist should be advised of LO/OVRAL® therapy when relevant specimens are submitted. 11. Susceptible women may experience an increase in blood pressure following administration of contraceptive steroids. 12. Cholestatic jaundice has been reported in users of oral contraceptives. If this occurs, LO/OVRAL® should be discontinued. This condition is more likely to occur in patients who have experienced cholestatic jaundice of pregnancy. Patients with a history of cholestatic jaundice of pregnancy should be carefully observed during LO/OVRAL® therapy.

ADVERSE REACTIONS OBSERVED IN PATIENTS RECEIVING ORAL CONTRACEPTIVES: A statistically significant association has been demonstrated between use of oral contraceptives and the following serious adverse reactions: thrombophlebitis, pulmonary embolism, cerebral thrombosis and hemorrhage, gall bladder disease.

Although available evidence is suggestive of an association, such a relationship has been neither confirmed nor refuted for the following serious adverse reactions: neuro-ocular lesions, e.g., retinal thrombosis, optic neuritis and hepatic lesions with or without intra-abdominal bleeding.

The following adverse reactions are known to occur in patients receiving oral contraceptives: nausea, vomiting, gastrointestinal symptoms (such as abdominal cramps and bloating), breakthrough bleeding, spotting, change in menstrual flow, amenorrhea during and after treatment, edema, chloasma or melasma, which may persist, breast changes: tenderness, enlargement and secretion, change in weight (increase or decrease), changes in cervical erosion and cervical secretions, suppression of lactation when given immediately post-partum, cholestatic jaundice, migraine, rash (allergic), rise in blood pressure in susceptible individuals, mental depression.

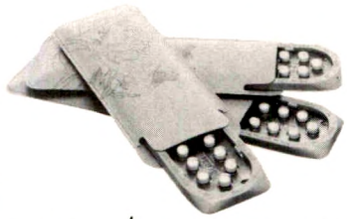
Although the following adverse reactions have been reported in users of oral contraceptives, an association has been neither confirmed nor refuted: anovulation post treatment, which tends to occur more frequently in women with a history of menstrual disorders, premenstrual-like syndrome, changes in libido, changes in appetite, cystitis-like syndrome, headache, intolerance to contact lenses, nervousness, dizziness, fatigue, backache, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, itching and vaginitis.

The following laboratory results may be altered by the use of oral contraceptives: hepatic function; increased sulfobromophthalein retention and other tests; coagulation tests; increase in prothrombin, Factors VII, VIII, IX and X; decrease in antithrombin III; increase in platelet aggregability; thyroid function: increase in PBI, and butanol extractable protein-bound iodine and decrease in T^3 uptake values; metyrapone test; and pregnanediol determination.

NOTE: For more specific details on LO/OVRAL, refer to package insert.

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OBSTETRICS

Improvement of faculty teaching performance in a department of obstetrics and gynecology by student evaluation

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The primary purpose of a faculty evaluation program is to upgrade the over-all quality of teaching and to identify the strengths and weaknesses of the individual teacher. Effective medical educators can thereby be suitably identified by objective and subjective criteria. Improvement of faculty teaching performance enhances student learning and can improve recruitment into our discipline.

WITHIN RECENT YEARS, there has been an increased awareness by faculty and students of the need for improvement in the delivery of medical education. Student pressures with faculty acquiescence, although sometimes reluctant, have fostered revision of traditional curricula, introduced selective and elective time for pursuit of special interests, restructured laboratory exercises, encouraged the development and use of self-instructional material, and promoted more exposure to small group teaching. Although these efforts would appear to be of educational benefit, results of the evaluation systems built into these new programs are presently awaited.

Likewise, the medical educator is now under scrutiny by the school administration, departmental chairman, colleagues, and the students he teaches. Traditionally, a teacher of medical students should have a strong academic orientation in the clinical or preclinical sciences, with specialized training perhaps in one area of his discipline. He should communicate his ideas with facility and assist the students in the acquisition of medical knowledge, provide suitable motivation, and develop the autonomy of self achievement. Medical education has developed over the years in accordance

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Table I. The evaluation form in current use broken down into its seven categories

Planning for class and organization
1. The instructor clearly stated the objectives of the course or the seminar at the outset.
3. The instructor's presentation was disorganized and hence hard to follow.
8. The instructor organized the material in a logical sequence.
22. The instructor stressed basic problems and restated issues to prevent the group from losing sight of the objectives.
23. The instructor allowed ample time for questions.
30. The instructor summarized the most important points periodically.

Communication
4. The instructor spoke too fast and was hard to follow.
5. The instructor used inappropriate technical jargon.
6. The instructor made an effort to clarify terms to avoid misunderstanding.
11. The instructor responded to questions in a concise and clear manner.
14. The presentation was suited to the student's level of sophistication.

Teaching personality (cognitive)
12. The instructor's presentation indicated a thorough knowledge of his subject.
18. The instructor's biases interfered with his presentation.
20. The instructor seemed quite impressed with his own knowledge.
26. The instructor monopolized the course (or seminar) by exhibiting his brilliance.
28. The instructor at times gave the impression that he was trying to bluff his way through a point he didn't fully understand.
32. The instructor would not admit not knowing the answer to a question.

Teaching personality (affective)
7. The instructor displayed sincere interest in his subject.
16. The instructor is available for consultation outside of the course (or seminar).
27. The instructor was free of disturbing mannerisms.
29. The instructor showed sincere interest in students.
33. The instructor was courteous.
35. The instructor's attitude toward patients seemed compatible with your concept of how a physician should behave.

Motivation
2. The instructor began teaching by arousing interest in the subject.
15. The instructor posed problems for students to solve.
21. The instructor made a real effort to stimulate students to think.
25. The instructor invited student comments instead of providing all the answers.
36. The instructor was so stimulating that you began to consider a career in his specialty.

Instructional techniques or methods
9. Instructional methods were in keeping with the announced objectives.
17. The instructor used audiovisual aids in a manner that detracted from his presentation.
24. The use of additional teaching aids would have provided a better learning experience.
38. The instructor relied excessively on audiovisual teaching aids.
39. Patients were used in a manner to enhance the learning experience.

Subject or content
10. The content was compatible with what has been learned from other courses and from readings.
13. The instructor's presentation was merely a repetition of the textbook.
19. The instructor presented divergent viewpoints for contrast and comparison.
31. The content was relevant to general medical education.
34. The material presented by the instructor will be a building block for future learning experiences.
37. The material presented by the instructor has relevance to your future career.

with individualistic philosophies and with a variation of experiences. Some highly creative educators show a preference for complexity and novelty with an interest in learning situations that require complicated resolutions, and others place a higher value on practical matters and utility. Some in medical education are oriented to achievement of their students and others to their own academic advancement.

At the New York Medical College a project was undertaken to improve teaching performance by faculty evaluation and subsequent teacher training. The enhancement of effective student learning was the primary goal of this research effort in medical education.

Background

A pilot project was started in 1969 to achieve more effective faculty performance in education by facilitating the acquisition of special teaching skills, knowledge, and attitudes which could then be translated by actions into effective student learning. A Medical Education Committee consisting of five full-time members of the faculty (Obstetrics-Gynecology, Pediatrics, Urology, and Internal Medicine) was chosen by the Dean because of their stated common interests in medical education, their motivation to devote time to the project, and their willingness to use their learning to improve instruction in their respective departments. The initial project was designed to evaluate the

Table II. Ratings of individual instructors by students and departmental chairman

	Ratings									
	Student					*Chairman*				
Category	*01*	*02*	*03*	*04*	*05*	*01*	*02*	*03*	*04*	*05*
Organization and clarity	2.38(5)	2.26(4)	1.61(1)	1.86(3)	1.73(2)	2.11(4)	2.22(5)	1.11(2)	1.22(3)	1.00(1)
Instructional methods	2.71(5)	2.14(3)	2.01(1)	2.08(2)	2.20(4)	2.57(5)	2.29(4)	1.86(2)	2.00(3)	1.57(1)
Teaching attitude	2.23(5)	2.07(3)	1.44(1)	1.95(2)	2.09(4)	1.33(4)	1.50(5)	1.00(2)	1.00(2)	1.00(2)
Motivation	2.85(5)	2.44(4)	1.83(1)	2.15(2.5)	2.15(2.5)	2.00(5)	1.50(4)	1.17(3)	1.00(1.5)	1.00(1.5)
Teaching approach	2.07(3)	2.45(5)	1.96(1)	2.10(4)	2.06(2)	1.30(1)	1.50(2.5)	1.50(2.5)	1.70(4)	2.00(5)
Over-all	2.45(5)	2.27(4)	1.77(1)	2.03(2)	2.05(3)	1.86(5)	1.80(4)	1.33(2)	1.38(3)	1.31(1)

teaching skills of individual faculty members and to use these data to help these instructors improve their teaching ability by highlighting not only their weaknesses but their strengths.[1–3]

Over a period of many months the Committee discussed the criteria which contribute to effective teaching and recorded a total of several hundred positive and negative factors. Ultimately, the most critical factors were put into question form and a total of 39 such questions evolved (Table I). Seven key aspects of teaching: planning and organization, communication, cognitive teaching personality, affective teaching personality, motivation, instructional techniques or methods, and subject or content, were appropriately distributed in the questionnaire. Since it is the learner himself who judges how best he learns, it was thought appropriate to have the principal evaluation of teaching skills evaluated by the recipient of that knowledge—the medical student.

At each phase of the development of the evaluation form, trials were run by having randomly selected members of the Junior class evaluate volunteer faculty participants. The questionnaire included a five-point scale multiple choice response, ranging from "strongly agree" to "strongly disagree" (or vice versa if the statement was negatively worded). During the pretest stages, efforts were made to ascertain the validity, accuracy, and reproducibility of the evaluation form. Use of informal faculty evaluation forms previously developed by our students correlated extremely well with the evaluation forms presently developed.

Each of the question responses was fed into the computer in a manner to yield a print out of the responses according to the seven key aspects of teaching previously mentioned.

Methods

All faculty participation was voluntary and an evaluation coordinator for the program was designated by each departmental chairman. These coordinators were responsible for describing the evaluation program to their departmental colleagues and for the distribution and collection of the forms. All students were briefed as to the objective of the evaluation—improvement of student learning by improvement of teaching. At the completion of each participating teacher's exposure to the students, the evaluation form was filled out anonymously and forwarded to the Medical Education Committee and sent to the computer center for analysis.

The entire Junior class, consisting of 135 students, were respondents. Five members of the Department of Obstetrics and Gynecology were evaluated by at least 40 students. Based on the responses of all students participating, an average score for each topic and an average score for all five topics were derived for each instructor, giving a set of six "student rankings." Each instructor was then ranked, within their respective departments, by topic score and over-all score.

As a corollary to this original study, the chairman was asked to cooperate in a comparative study. The chairman was asked to complete a rating sheet for those instructors in his department previously evaluated by the student. As previously computed for the student evaluations, there was a set of six "chairman rankings." Additionally, the chairman was asked to rank his instructors by usual criteria, without considering the detailed questionnaire ("chairman sequence").

Results

The Chairman of Obstetrics and Gynecology's ranking agreed with the student identification of the two lowest ranked instructors (Table II). Although the three highest rated instructors were identified by both students and chairman, there was no agreement in their place rank.

A comparison of the ratings by students and chairman of the over-all teaching performance of the instructors evaluated (Table III) indicates that the chairman consistently rated his faculty better.

Table IV indicates that for all of the categories

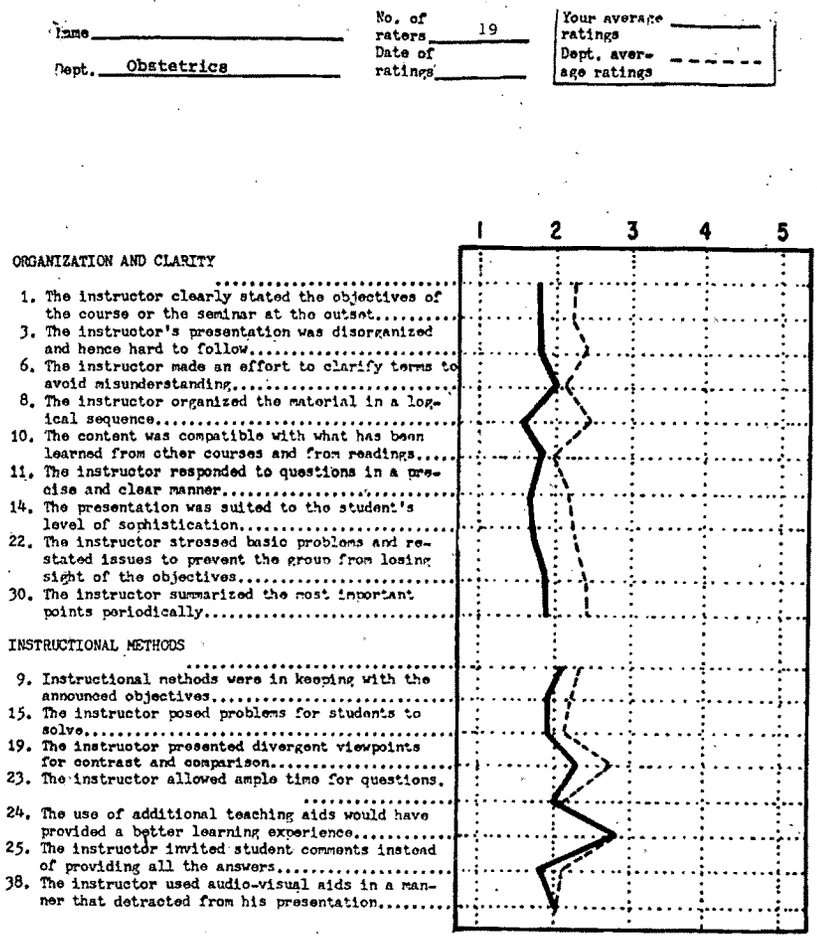


Fig. 1. Evaluation scores and instructor profiles.

evaluated and for the over-all score none of the correlations between rankings of instructors as derived from students and those by the chairman was significant at the 0.05 level.

Comment

Any teacher evaluation program can be considered threatening to many members of a faculty, and the major objective of improvement of teaching skills must be stressed.[4] Likewise, evaluation scores and instructor profiles (Fig. 1) should be kept confidential and made available only to the individual instructor. From our experience it would appear that the majority of our departmental faculty was eager to cooperate in this evaluation.

Within recent years the qualities of a medical educator have assumed equal importance in judging a faculty member's contribution to his department, whereas previously only research potential, clinical ability, number of grants, and publications were considered. Unfortunately, there are only two ways to judge teaching through the medium of the students[5]: The objective criterion is based on what students have learned from the teacher, and the subjective criterion is based on student evaluations of teacher effectiveness. In 1960, 40 per cent of institutions of higher learning engaged in student evaluations of instructors and in the 1970's this should increase.[6] With few exceptions, medical schools have lagged behind in the adoption of systematic student evaluation of faculty. Student evaluation is but one means of assessing an individual's teaching skills and can form an integral part of an instructor's teaching profile. This profile can be utilized in the pinpointing of strengths and weaknesses in an individual's teaching capabilities.

Our initial studies show some disagreement between the departmental chairman and student evaluation of "teacher effectiveness." There were differences as well between the "chairman rankings" and "chairman sequence." A chairman's over-all appraisal of a faculty member includes more factors than his evaluation of that person as a teacher. A hard-working, productive, nondemanding faculty member may be rated very highly by the chairman, yet he may not be an

Table III. Cumulative ratings by students and chairman of instructors' teaching performance

Category	Ratings	
	Students	*Chairman*
Organization and clarity	1.97	1.53
Instructional methods	2.23	2.06
Teaching attitude	1.96	1.17
Motivation	2.28	1.33
Teaching approach	2.12	1.60
Over-all	2.11	1.54

Table IV. Correlation* of ratings of instructors by students

Category	*A*†	*B*‡	*C*§
Organization and clarity	0.800	0.600	0.900
Instructional methods	0.400	0.100	0.800
Teaching attitude	0.500	0.100	0.900
Motivation	0.700	0.575	0.875
Teaching approach	0.075	0.500	−0.775
Over-all	0.700	0.500	0.800

*Spearman rank order correlation.

†Correlation of ratings of instructors by students with those by chairman.

‡Correlation of ratings of instructors by students with chairman's sequences.

§Correlation of chairman's sequences assigned to instructors by chairman with chairman's rating.

outstanding teacher. The differences between student and chairman rankings may be due to several factors. The chairman may not adequately audit the teaching sessions of his faculty and be aware of the strengths and weaknesses of his instructors. The fact that the chairman is usually a teacher himself creates a natural bias and he cannot view his faculty through the eyes of students who base their evaluations on teaching skills and not on objective test results.

Phase 2 of this project is the discussion of the "profile" with each instructor evaluated by the Medical Education Committee. The participating teacher is invited to meet with the Committee to review his profile and methods for self-improvement will be discussed. Some faculty members may feel uncomfortable discussing their educational strengths and weaknesses with colleagues and every effort is made to overcome this problem through mutual trust, freedom from coercive or disciplinary potential, and confidence.

Table V. Effect of profile on teaching performance

Rating	Instructor				
	I	*II*	*III*	*IV*	*V*
Before profile					
02	2.26	2.14	2.07	2.44	2.43
04	1.86	2.08	1.95	2.15	2.10
05	1.73	2.20	2.09	2.15	2.06
After profile					
02	1.95	1.87	1.82	1.91	2.06
04	1.77	2.05	1.75	1.94	2.20
05	1.73	1.98	1.78	1.99	2.13

Of interest, however, is that the evaluated individual's review of his teaching profile appears to provide motivation for self-improvement, without formal remedial techniques. This is evident in the improvement in the profile of instructors (02, 04, 05) after receipt of their profile and student re-evaluation (Table V).

The primary goal of our department is educaton, undergraduate and graduate, and teaching in our department is done by senior faculty members (including the chairman), selected for their skills and ability to impart the knowledge that they have. If a faculty member is ineffective as a teacher, he is not given teaching assignments no matter what his rank or research achievements. This constant re-evaluation of faculty teaching in our department has improved student learning and is a major factor in our success in student recruitment into our specialty.[7]

The primary purpose of a faculty evaluation program is to upgrade the over-all quality of teaching in the medical school and to identify the strengths and weaknesses of the individual teacher. Ancillary benefits of teacher evaluation is the ability by objective and subjective criteria to identify effective medical educators and reward them in similar fashion to prolific writers, grant recipients, and productive researchers. The provider and consumer in medical education must find some common ground for the definition and measurement of good teaching. Until this occurs, there is little chance for improvement in learning experience and effectiveness.

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Changes in evolution of free epinephrine, norepinephrine, and vanillylmandelic acid in the urine of rabbits during late pregnancy and parturition

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The present study describes evolutionary changes in urinary excretion of epinephrine, norepinephrine, and vanillylmandelic acid during pregnancy and parturition. Both the catecholamines and their metabolite were determined in 24 hour urine samples of rabbits from day 13 of pregnancy. During 13 and 28 days of gestation, urinary excretion of epinephrine was at its lowest value. As compared to normal rabbits, an increase of 135 per cent in epinephrine excretion during day 30 of pregnancy was observed. Norepinephrine excretion reached its maximum level during day 13 but constant declines occurred afterward. Contrary to epinephrine, urinary excretion of norepinephrine was at the highest value days 23 and 30 of pregnancy. After day 19 vanillylmandelic acid declined continuously up to day 30. During parturition vanillylmandelic acid and norepinephrine excretion returned to the control values but epinephrine remained slighly higher. These alterations in catecholamine urinary excretion have been suggested as a consequence of modified endocrine and sympathetic activity.

THE PRESENCE and physiologic role of biogenic amines and their metabolites has been known for several years.[1] The importance of catecholamines during pregnancy has been considered mainly to be of interest in the diagnosis of pheochromocytoma.[2] Cyclic changes in concentration of uterine epinephrine were observed earlier.[3–5] Adrenergic receptor activity of catecholamines in gestational myometrium also varies to a great extent.[6–8] Very few studies have been performed to determine the influence of pregnancy and parturition on urinary excretion of epinephrine and norepinephrine.[9–11] Most of the past observations are highly speculative and limited to human beings. Among all the investigators, Zuspan[11] was the only one who determined the excretion of epinephrine and norepinephrine in urine samples collected for 24 hours. He noticed increased excretion of both epinephrine and norepinephrine during the first 24 hours following delivery but only the increase in norepinephrine excretion was statistically significant. Our recent paper shows important changes in urinary catecholamine levels throughout pregnancy in rats. We also observed statistically significant increases in plasma epinephrine on days 30 and 31 of gestation in rabbits.[13] The present study was designed to determine pregnancy-induced alterations in urinary excretion of catecholamines. Parallel determinations of urinary vanillylmandelic acid were also made. Such a study has not been performed previously in rabbits.

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Methods

Albino rabbits of New Zealand strain were utilized throughout the experiments. The females were made pregnant by keeping one male with one female for 1 hour under constant observation. After copulation, the females were separated immediately and kept in individual stainless steel cages. They were verified for pregnancy by palpation 14 days after copulation. Under normal conditions parturition occurred between days 31 and 32 of pregnancy.

Collection of urine. Twenty-four hour urine was collected by placing one pregnant rabbit in an indi-

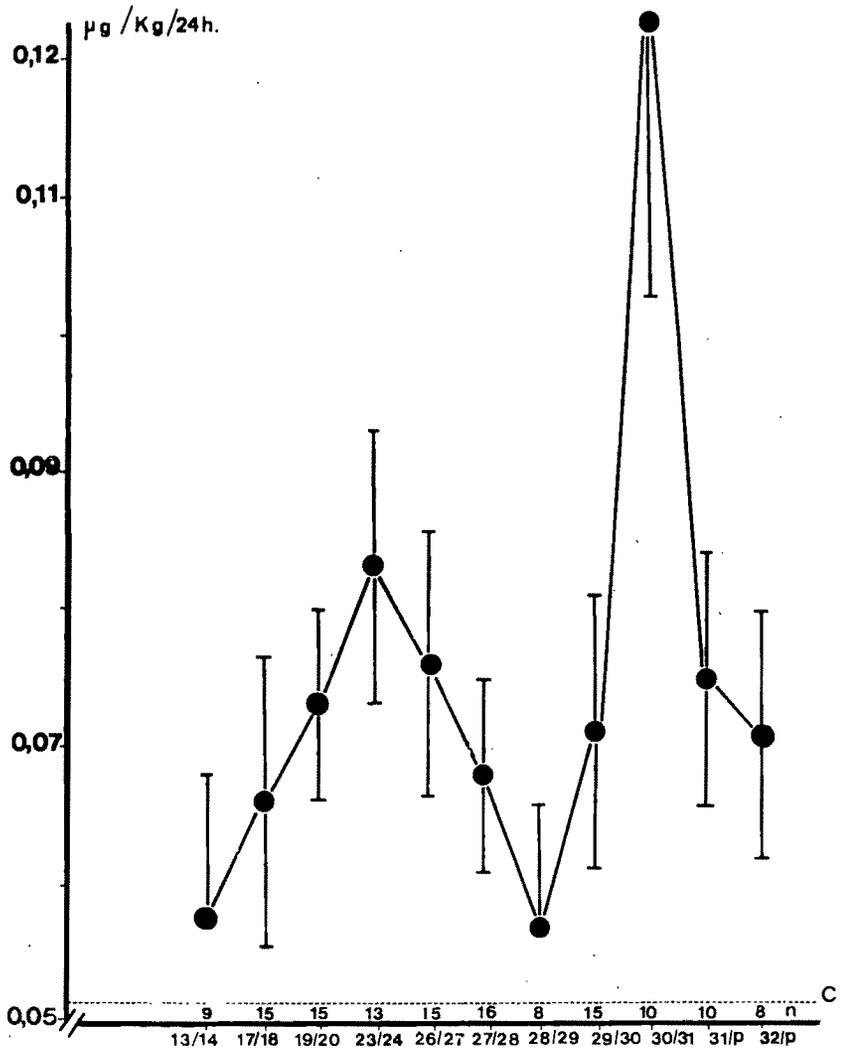


Fig. 1. Urinary excretion of free epinephrine in micrograms per kilogram of body weight per 24 hours in pregnant rabbits from the thirteenth day of gestation to the end of parturition. *C*, Nonpregnant value; *n*, number of determinations.

Statistical differences between:

13/14 and 23/24	$P < 0.05$
23/24 and 28/29	$P < 0.05$
13/14 and 30/31	$P < 0.005$
28/29 and 30/31	$P < 0.005$
30/31 and 31/P	$P < 0.001$
30/31 and 32/P	$P < 0.001$

vidual metabolic cage. The surrounding walls of the metabolic cages were made of high-quality stainless steel. The bottoms of the cages which were open were equipped with Armodur funnels (special synthetic material which does not interfere with trihydroxindole procedure). Special separating discs made of Armodur were placed on the top of the funnels. Both the funnels and the discs were well rinsed with twice-distilled water and finally with 2N HCl. This procedure prevented the urine from being in contact with the metallic part of the metabolic cages and also provided for the separation of feces from urine. The urine was collected in 500 ml. Pyrex flasks containing 5 ml. of 5N HCl. Throughout urine collection, the temperature was maintained at 20° C.

Separation of urinary catecholamines. Epinephrine and norepinephrine were separated by their adsorption on acid-activated aluminum oxide.[14] The batch

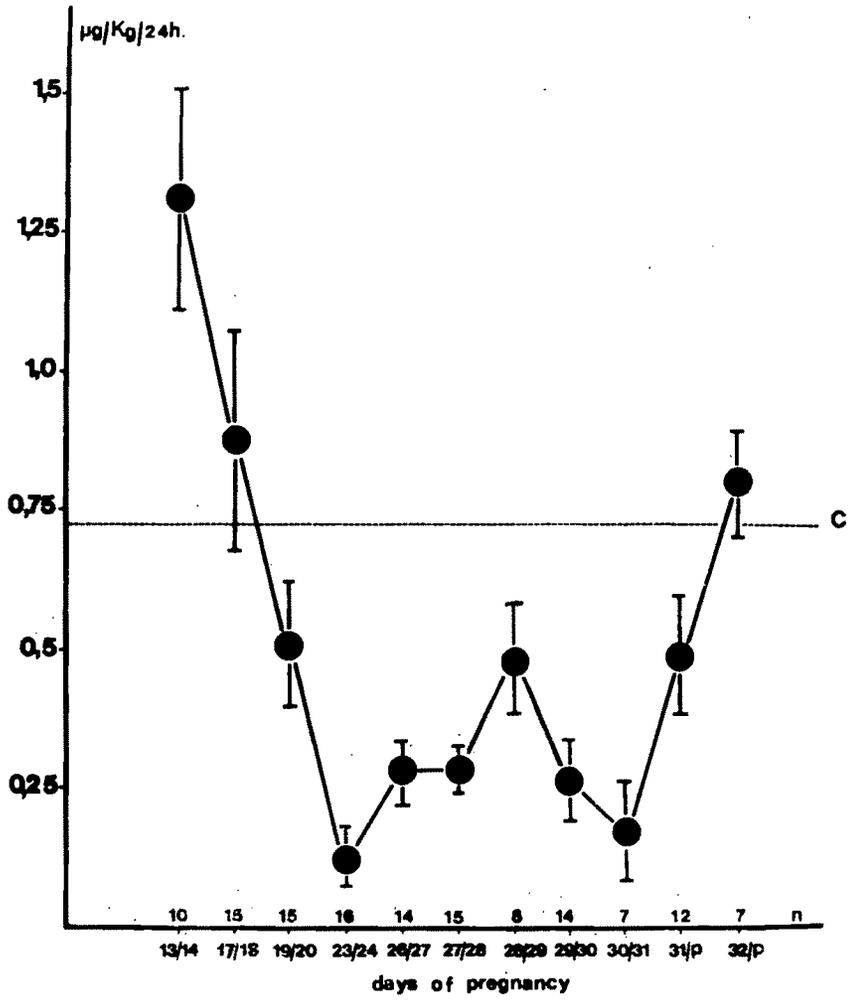


Fig. 2. Alterations in excretion of free norepinephrine in urine of rabbits from thirteenth day of pregnancy to parturition. *C*, Norepinephrine in nonpregnant rabbits; *n*, number of cases.

Statistical differences between:

13/14 and 19/20	$P < 0.005$
13/14 and 23/24	$P < 0.001$
19/20 and 23/24	$P < 0.005$
13/14 and 26/27	$P < 0.001$
19/20 and 27/28	$P < 0.05$
23/24 and 28/29	$P < 0.001$
28/29 and 30/31	$P < 0.02$
30/31 and 31/P	$P < 0.025$
30/31 and 32/P	$P < 0.001$
31/P and 32/P	$P < 0.025$

process was preferred to column chromatography.[16] The acid-activated aluminum oxide was well rinsed with 2 × 25 ml. twice-distilled water. The urine was mixed and filtered and 25 ml. of it was adjusted to pH 8.4 by addition of different concentrations of NaOH solution (0.2 to 2N). The urine at pH 8.4 was transferred to a beaker containing 1 Gm. of washed aluminium oxide and stirred constantly. The pH was maintained at 8.4 by a direct-reading glass electrode pH meter. After 5 minutes of stirring, the urine was centrifuged for 1 minute at 5,000 × g and the supernatant was discarded. The alumina was washed with 15 ml. of twice-distilled water and centrifuged and the supernatant was discarded. Epinephrine and norepinephrine were eluted with addition of 4 ml. of 0.25N acetic acid.[16] After thorough mixing the acid supernatant was separated from the alumina for fluorometric estimation of catecholamines. The total

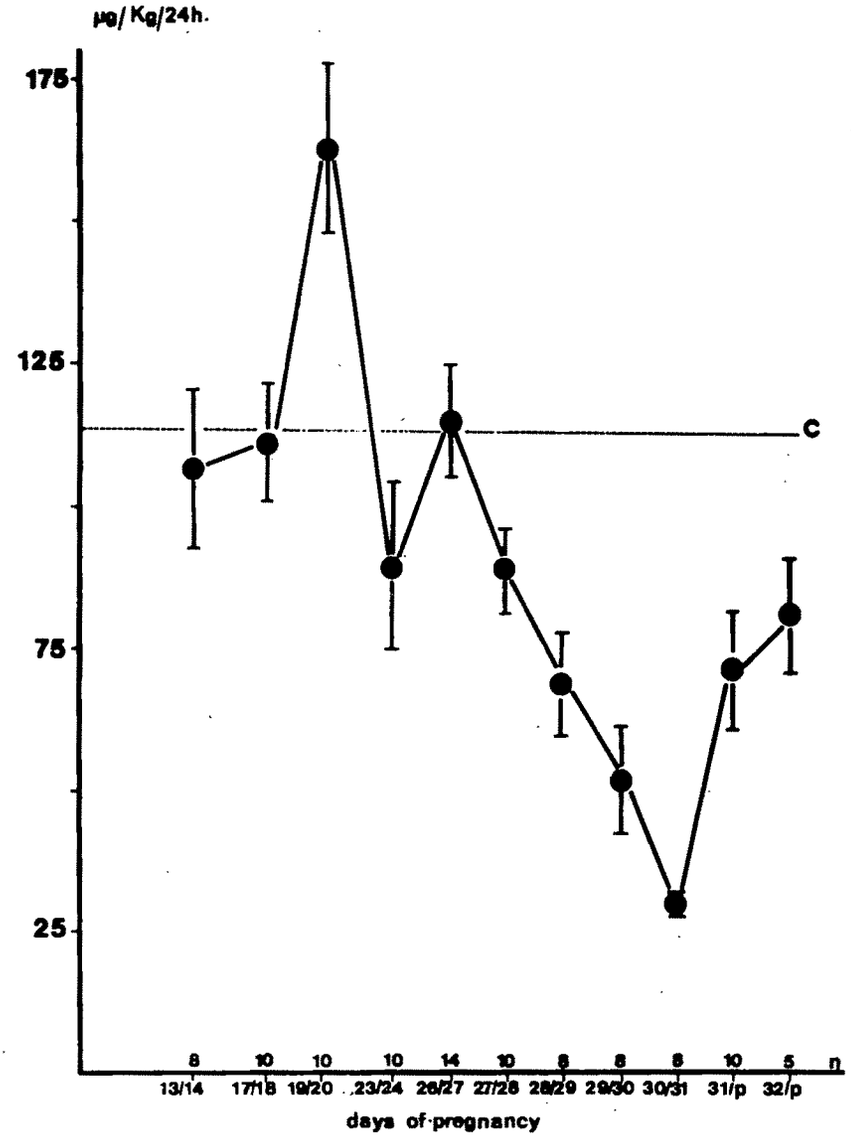


Fig. 3. Evolution of vanillylmandelic acid in urine of pregnant rabbits from thirteenth day of gestation to the end of parturition. *C*, Control value; *n*, number of animals used for each group.
Statistical differences between:

13/14 and 19/20	$P < 0.01$
19/20 and 23/24	$P < 0.005$
19/20 and 26/27	$P < 0.02$
19/20 and 28/29	$P < 0.001$
26/27 and 28/29	$P < 0.005$
28/29 and 30/31	$P < 0.001$
30/31 and 31/P	$P < 0.005$
30/31 and 32/P	$P < 0.001$

time of operation ranged between 7 and 8 minutes.

Fluorometric assay of catecholamines. The method of Euler and Lishajko[16] utilizing the trihydroxindole reaction was applied. Fluorescence was measured with an Eppendorf apparatus. The results are expressed in micrograms per kilogram of body weight per 24 hours. This method of expressing results for urinary excretion of catecholamines has been widely employed by von Euler and many others.[17–20] The later authors adopted this method of expressing results to compare catecholamine excretion in many different species. Recently some correlation between body weight and

Table I. Alterations in urinary excretion of catecholamines and their metabolite during pregnancy in rabbits

Day of pregnancy	*E + N**	*E + N + VMA*	*Increase or decrease in N from controls (%)*	*Increase or decrease in E from controls (%)*	$\frac{E}{E+N}$
13/14	1.37	107.37	+77	+4	0.042
17/18	0.95	112.95	+19	+27	0.07
19/20	0.58	162.58	−32	+40	0.125
23/24	0.21	88.21	−83	+60	0.397
26/27	0.36	115.36	−62	+46	0.211
27/28	0.36	87.36	−61	+31	0.192
28/29	0.54	68.54	−36	+10	0.106
29/30	0.34	51.34	−64	+34	0.21
30/31	0.3	30.3	−76	+135	0.41
31/P	0.25	71.25	−34	−44	0.3
32/P	0.87	81.87	+8	−34	0.08

*E, Epinephrine; N, norepinephrine; VMA, vanillylmandelic acid. The total excretion of free E and N as well as E + N + VMA have been provided to evaluate total changes. The percent increases in E and N calculated from their respective control values show the influence of one catecholamine on another one. E(E + N) is a rough estimation of rate of norepinephrine conversion to epinephrine.

catecholamine excretion has been observed. Therefore, catecholamine excretion in micrograms per kilogram per 24 hours takes into account all the changes possibly induced by variations in body weight from one animal to another, especially during late pregnancy.

Assay of vanillylmandelic acid. Vanillylmandelic acid was assayed spectrophotometrically by its conversion to vanillin with several modifications of the previous techniques.[21, 22] A 5 ml. sample of urine was used for each determination. Final extraction was made in 4 ml. of potassium carbonate to avoid the use of micro cells. The optical density was measured on a Beckman DBT spectrophotometer at 360 mμ. The extraction of vanillylmandelic acid was linear within concentrations of 10 to 150 μg. The details of the method are published elsewhere.[23]

Statistical analysis was performed according to the method of Fisher, utilizing Student's t test. The means are expressed with standard errors.

Results

Fig. 1 illustrates the evolutionary changes in urinary epinephrine in micrograms per kilogram per 24 hours from day 13 of pregnancy to parturition. Constant increases in epinephrine excretion were observed up to days 23/24 of pregnancy. At days 23/24 urinary epinephrine was markedly higher than on days 13/14. Afterward, constant declines occurred up to days 28/29. During days 28/29 epinephrine was at its lowest value. This decline was followed by increased epinephrine output on days 29/30 and 30/31. Urinary excretion of epinephrine reached its maximum value during days 30/31 of pregnancy. Parturition at day 31 or 32 showed constant declines in excretion of this hormone.

The alterations in urinary excretion of norepinephrine during last part of pregnancy and parturition are shown in Fig. 2. Urinary norepinephrine demonstrated continuous declines in its excretion during days 13/14 to 23/24 of pregnancy. At days 23/24 urinary epinephrine was tenfold lower than on days 13/14. The excretion started to rise afterward up to days 28/29. These increases were followed by constant declines again to days 30/31. At parturition on day 31 or 32 the output of norepinephrine again started to increase. At parturition norepinephrine excretion was nearly the same as in the nonpregnant rabbits.

Fig. 3 shows the evolutionary pattern of vanillylmandelic acid in the 24 hour urine of pregnant rabbits during days 13/14 of pregnancy to parturition. During days 13/14 and 19/20 of pregnancy excretion of this catecholamine acid metabolite increased. At days 19/20 this value was more highly increased than on days 13/14. Except for a small increase on days 26/27 continuous declines in urinary excretion of vanillylmandelic acid were observed. At days 30/31 of pregnancy the excretion was 3½ times lower than on days 13/14. Similarly to epinephrine and norepinephrine, vanillylmandelic acid showed increases in its excretion during parturition at day 31 or 32.

Table I provides a comparison during different days of pregnancy in the total excretion of epinephrine + norepinephrine + vanillylmandelic acid. The ratio epinephrine/(epinephrine + norepinephrine), which is a rough evaluation of the rate of norepinephrine conversion to epinephrine, is also provided. The per cent increases or decreases in epinephrine and

norepinephrine as compared with their respective nonpregnant values are also given. These ratios or per cent changes in all the catecholamines provide a rapid comparison between different days of pregnancy. The maximum increase in epinephrine + norepinephrine excretion occurred during days 13/14 of urine collection. The total excretion of epinephrine + norepinephrine + vanillylmandelic acid increased to the maximum value on days 19/20 of pregnancy. Except for days 13/14, 17/18, and 32/P, the per cent increases in epinephrine excretion as compared with control values were followed by declines in norepinephrine excretion. These increases provide direct relationship of urinary epinephrine with urinary norepinephrine.

Comment

The results of the present investigation are in agreement with our previous findings about the accelerated release of catecholamines in plasma of rabbits on days 30/31 of pregnancy. Urinary epinephrine and norepinephrine are mainly the product of catecholamines in plasma as 4 per cent of the total blood catecholamines are excreted in urine.[24] In our previous paper we suggested that the enhancement of plasma epinephrine results from adrenomedullary secretion due to its direct influence on catecholamine storage in the adrenal gland.[13] The increase in adrenomedullary secretion can be considered as an important factor for the rise in urinary excretion of epinephrine as any change in plasma catecholamines would directly affect urinary content of both epinephrine and norepinephrine. The present findings also show the highest increase on days 30/31 of pregnancy in urinary output of epinephrine, which is the principal hormone of the adrenal medulla; at the same time a strong decline in urinary norepinephrine occurred. This observation suggests that the increased epinephrine excretion is not only induced by an increased rate of adrenomedullary secretion but also by accelerated synthesis of epinephrine from norepinephrine in the adrenals: when there is increased epinephrine synthesis, less norepinephrine is available for secretion from the adrenals.

The findings of Zuspan[11] about the urinary excretion of epinephrine and norepinephrine in pregnant and parturient women show important increases in the output of both the amines. He noticed nearly twofold and threefold increases in epinephrine and norepinephrine, respectively, 24 hours after delivery, but only norepinephrine urinary excretion showed statistically significant differences from the antepartum or postpartum values.[11] These clinical observations appear difficult to compare with urinary excretion of epinephrine and norepinephrine in rabbits. In the rabbit we observed the maximum increase in urinary excretion of epinephrine on days 30/31 of pregnancy, which is at least 24 to 48 hours in advance of parturition. This maximum increase in epinephrine before labor cannot be considered due to the stress observed during labor and delivery, just as severe muscular exercise, operation, and trauma also produce an increase in the output of these hormones.[25–27] The findings of a similar study in human beings, goats, rats, sheep, and cats are difficult to compare with our present observations as in that study the urine was hydrolyzed prior to catecholamine estimation and the results express the total amount of free and conjugated epinephrine and norepinephrine.[28] Moreover, the latter investigators expressed their results in micrograms per 24 hours without taking in account the differences in body weight from one subject to another.

Our extensive studies on catecholamines in plasma, urine, and tissues in the rat throughout the duration of pregnancy, parturition, and postpaturition provide evidence that hormonal factors play an important role in the altered pattern of catecholamine regulation in gestation.[12, 29, 30] The activities of enzyme monoamine degradation (monoamine oxidase and catechol-O-methyl transferase) are also very low during the last part of pregnancy.[31] These declines in activities of enzyme monoamine oxidase and catechol-O-methyl transferase could be related to concentration changes of corticosteroids, progesterone, and estrogens during pregnancy. All these hormones have been shown to exert an important action on the enzymes of norepinephrine synthesis and degradation.[32–34] The respective increases and and declines in urinary epinephrine and norepinephrine on days 23/24 of pregnancy also suggest that catecholamine biosynthesis was affected on these dates. This is further evidence in favor of our hypothesis of direct participation of endocrine function with the regulation of biogenic amines in pregnancy. Recently it has been shown that plasma and serum cortisol in rabbits increases to its maximum value on day 30 of gestation and this increase is followed by a sharp decrease at parturition.[35] Peripheral plasma levels of progesterone in the rabbit during pregnancy also fluctuate to a great extent and these fluctuations are specially marked during the last days of pregnancy.[36] The action of all these hormones on catecholamine biosynthesis, release, and metabolism is well established and supports our hypothesis of endocrine involvement.

The variations in norepinephrine excretion could be related to epinephrine as most of the increases in epinephrine were followed by decreases in norepinephrine (Table I). Since a major portion of norinephrine in blood is released from sympathetic nerves, these declines in norepinephrine excretion could also be attributed to a decline in activity of the sympathetic nervous system. The decreased excretion of vanillylmandelic acid throughout the gestation results from decreased activities of enzymes of catecholamine metabolism.[31] When the catecholamines are degraded at a lower rate, the metabolite formation is also low.

The observed alterations in epinephrine, norepinephrine, and vanillylmandelic acid suggest that the processes of catecholamine excretion and metabolism are under hormonal influence during last part of pregnancy in rabbits. Our results on glycogen storage during pregnancy and under hormone administrations suggest markedly important declines in hepatic glycogen throughout the last part of pregnancy and these differences could be correlated with catecholamine variations.[37] An important link between catecholamines and different steroid hormones is proposed. A comprehensive review of all possible interactions between catecholamines, progesterone, estrogens, and many other hormones has been elaborately analyzed by Zuspan and his collaborators.[38]

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Correlation between catecholamine content of the human Fallopian tube and the uterus and plasma levels of estradiol and progesterone

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The content of catecholamines in the Fallopian tube and the uterus and the plasma levels of estradiol and progesterone were studied in cycling women. During the follicular phase norepinephrine levels were 33.4 ± 7.1, 50.5 ± 8.0, and 145.7 ± 43.6 ng. per gram of wet tissue in the external, middle, and internal segments of the Fallopian tube, respectively. During the luteal phase norepinephrine content increased significantly in the external and middle portions (219.3 ± 57.0 and 206.2 ± 34.3 ng. per gram) whereas it remained unchanged in the internal one (185.2 ± 53.6 ng. per gram). The NE content of the external and middle segments correlated significantly with plasma progesterone levels (r = 0.76 and 0.82, respectively, whereas oviductal epinephrine levels did not show significant changes as a function of the stage of the menstrual cycle. Uterine epinephrine content decreased by 67 per cent during the luteal phase whereas norepinephrine remained unchanged.

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THE SIGNIFICANCE of the sympathetic nervous system in the regulation of the contractile activity of the oviduct has become apparent in recent years, since tubal dysfunction affects fertility.[1, 2] The smooth muscle of the Fallopian tube is innervated by adrenergic nerve fibers along its entire length.[3] Alpha- and beta-adrenergic receptors mediating changes in resting pressure[4] and motility[5] have been well demonstrated in human Fallopian tubes in vivo and in vitro. Norepinephrine, the sympathetic postganglionic neurotransmitter, is present in the circular muscular layer of the Fallopian tube, particularly in the isthmus, in which a high density of adrenergic terminals running parallel to the muscle fibers occurs. In addition, vascular sympathetic innervation has been demonstrated throughout the tube.[3]

Since the short adrenergic neurons innervating the genital tract exhibit functional properties that differentiate them from long adrenergic neurons,[6] a hypothesis can be postulated according to which these differences may be due in part to sex steroid action at the level of

Table I. Catecholamine content of the Fallopian tubes and peripheral plasma levels of estradiol and progesterone in normal cycling women

Catecholamine content (ng./Gm. wet tissue)						*Plasma steroids levels (pg./ml.)*	
Norepinephrine			*Epinephrine*				
External segment	*Middle segment*	*Internal segment*	*External segment*	*Middle segment*	*Internal segment*	*Estradiol*	*Progesterone*
Proliferative:							
20.2	30.5	85.2	36.7	37.8	69.5	73	590
31.3	67.7	94.9	59.5	56.5	58.8	73	590
18.6	20.3	66.7	30.9	6.9	12.2	105	375
18.4	34.7	60.7	19.6	36.7	139.2	265	123
64.1	66.5	369.5	52.0	86.7	23.1	265	123
27.9	71.6	237.8	36.4	79.8	169.2	172	870
56.5	62.2	105.5	73.1	78.6	21.7	244	797
33.4	50.5	145.7	44.0	34.7	56.2	171	495
±7.1*	±8.0	±43.6	±6.9	±11.0	±16.5	±33	±133
Secretory:							
104.9	147.9	65.6	15.6	23.3	5.2	218	2,445
47.0	55.1	47.1	51.3	2.3	6.4	218	2,445
72.1	135.3	84.6	29.0	51.2	40.8	343	2,576
99.7	218.14	99.7	69.0	105.2	160.2	259	3,120
236.8	265.5	86.3	67.3	109.3	60.1	187	3,429
411.6	343.8	363.3	30.8	53.0	181.0	187	3,429
330.5	310.5	399.4	36.5	70.0	74.5	294	3,387
451.9	173.2	335.9	185.4	26.7	41.4	294	3,387
219.3	206.2	185.2	60.6	55.1	71.2	249	3,027
±57.0†	±34.3†	±53.6	±19.0	±13.5	±23.3	±20†	±162†

*Mean ± S.E.M.

†Significantly different from the follicular phase.

the target organ. In the rabbit oviduct, for instance, norepinephrine levels are modified by the administration of estradiol or progesterone.[7] This article deals with the modifications in norepinephrine and epinephrine levels of different portions of the human Fallopian tube and the uterus as a function of the plasma levels of estradiol and progesterone. It will be shown that a regional distribution of catecholamines exists in the human tube and that norepinephrine content exhibits changes during the menstrual cycle which correlate with plasma progesterone levels.

Materials and methods

Tissues. Fallopian tubes were obtained from patients having a salpingectomy at the same time as hysterectomy. The tubes were divided into three zones as follows: (1) *internal,* the short portion (23 to 25 per cent) with a narrow lumen of about 0.2 to 0.5 mm. lying distal to the fundus; (2) *middle,* the longest portion (61 to 62 per cent) with its ever-widening lumen, from 1 to 2 mm. at the isthmus to 2 cm. near the distal end; (3) *external or infundibulum,* the distal portion terminating in the fimbriated end (13 to 14 per cent). In addition, myometrial and plasma samples were also taken.

Assays. Catecholamine concentration was measured spectrofluorometrically, as described by Laverty and Taylor,[8] after isolation by adsorption onto alumina at pH 8.6.[9] Internal and external standards were used in order to correct procedure losses. Recovery from alumina columns averaged 74 per cent.

Plasma progesterone and estradiol levels were determined by a double-antibody radioimmunoassay method. To measure progesterone, an antiserum produced in rabbits in response to 11-α-hydroxyprogesterone hemisuccinate-bovine serum albumin (BSA) was used.[10] Plasma diethylether extracts were processed in triplicate. Goat antirabbit gamma-globulin was utilized for separation of bound and free fractions. A standard curve was constructed for each assay with unlabeled progesterone at nine dose levels to give a range from 5 to 1,500 pg. Results were expressed in picograms per milliliter of plasma. Specificity of the antiserum was assessed by incubation with unlabeled 17-α-hydroxyprogesterone, 20-β-hydroxyprogesterone, pregnenolone, and corticoids. All the studied steroids failed to cross-react up to a maximal concentration of 10 ng. Sensitivity = 10 pg. could be distinguished from 0. The assay recovery was 88.0 ± 5.4 per cent.

Table II. Catecholamine content of the uterus and peripheral plasma levels of estradiol and progesterone in normal cycling women

Catecholamine content (ng./Gm. wet tissue)		*Plasma steroid levels (pg./ml.)*	
Norepinephrine	*Epinephrine*	*Estradiol*	*Progesterone*
Proliferative:			
84.2	62.2	73	590
31.3	29.0	105	375
163.4	31.0	265	123
65.6	30.7	172	870
113.8	64.5	244	797
91.7	43.5	172	552
±22.3*	±8.1	±37	±136
Secretory:			
76.2	12.9	218	2,445
107.9	10.3	343	2,576
129.4	29.9	259	3,120
76.7	12.0	187	3,429
73.1	7.9	294	3,387
92.6	14.6	260	2,991
±11.1	±3.9†	±27†	±146†

*Mean ± S.E.M.
†Significantly different from the follicular phase.

To measure estradiol, antiserum of Wein Laboratories (Succasunna, New Jersey) was utilized. Extracts of plasma were purified by thin-layer chromatography and 25 pg. could be clearly distinguished from 0. The assay recovery was 63.0 ± 6.7 per cent.

Results

The levels of epinephrine and norepinephrine in different portions of normal human Fallopian tubes as a function of plasma levels of estradiol and progesterone are shown in Table I.

Despite the great variance in norepinephrine content among individual samples, a definite pattern of distribution could be established. During the proliferative phase of the menstrual cycle, norepinephrine levels of the internal portion of the Fallopian tube were two to threefold those of the external and middle portions. No differences were observed between the external and the middle segments. High levels of plasma progesterone and a four to sixfold increase in norepinephrine content of the Fallopian tube in the external and middle segments were observed in the luteal phase. In contrast, no significant changes were detected in the internal oviductal segment (Table I). Norepinephrine content of the external and middle portions of the human Fallopian tube correlated significantly with plasma progesterone (Figs. 1 and 2) but not with plasma estradiol. In addition, no correlation was observed between tubal norepinephrine content and plasma estradiol, nor between epinephrine

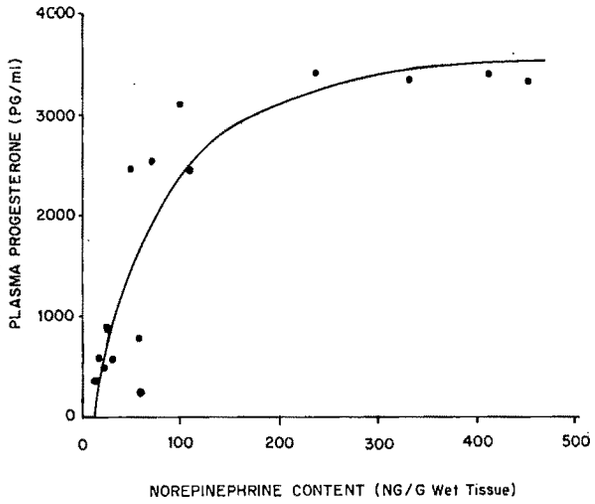


Fig. 1. Correlation between norepinephrine content of the external segment of the human Fallopian tube and plasma levels of progesterone. Curves were fitted with a computer, following Seidel's[18] method of successive approximations (r = 0.77; n = 15).

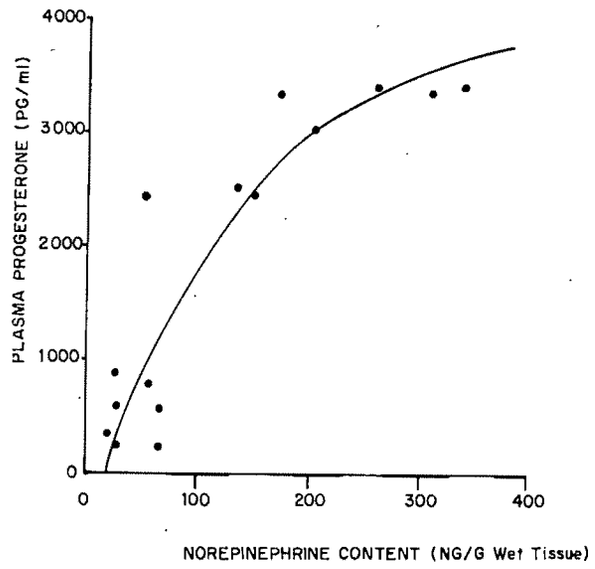


Fig. 2. Correlation between norepinephrine content of the middle segment of the human Fallopian tube and plasma levels of progesterone. Curves were fitted with computer, following Seidel's[18] method of successive approximations (r = 0.87; n = 15).

ratios and plasma estradiol and progesterone levels. Likewise, epinephrine content did not show significant changes as a function of the stage of the menstrual cycle in any of the segments of the Fallopian tubes.

Table II shows the levels of epinephrine and norepinephrine of the human myometrium during the menstrual cycle. Epinephrine content decreased sig-

nificantly during the luteal phase whereas norepinephrine remained unchanged. Correlation between epinephrine and plasma progesterone levels approached significance ($r = 0.68$; $n = 10$).

Comment

The foregoing results indicate that the human Fallopian tube exhibits regional differences in norepinephrine content concomitant with the fluctuation in progesterone levels. During the follicular phase norepinephrine concentration of the internal segment of the tube was two to three times greater than that of the external and middle portions. After ovulation and concomitantly with a functional corpus luteum, a rise in neurotransmitter content occurred in the external and middle segment of the tube, whereas norepinephrine content of the uterine portion of the tube did not change appreciably.

Owman and associates[11] reported regional differences in the concentration of norepinephrine in human Fallopian tubes resembling those observed in the present work during the follicular phase, i.e., norepinephrine levels of the internal portion of the human oviduct were significantly larger than those of the remaining tube. They failed, however, to detect cyclic changes in the total content of the catecholamine of the tube; no information was provided on cyclic changes in the regional distribution of norepinephrine nor on plasma estradiol or progesterone levels.

The functional significance of the changes in oviductal neurotransmitter content is still obscure. In rabbits, insemination and HCG treatment resulted in a significant fall in norepinephrine levels of the isthmus, whereas administration of estrogen or progesterone in doses adequate to disrupt egg transport induced increases in isthmic neurotransmitter content.[7] These observations were interpreted to suggest that a normal passage of eggs requires low levels of norepinephrine in the isthmus.

Both estrogens and progesterone are relevant entities in the control of ovum transport in the oviduct. It is widely accepted that estrogens stimulate and progesterone represses spontaneous oviductal contractile activity.[1, 2] The predominant response to catecholamines of isolated isthmic muscle obtained from the estrogenic phase in women and rabbits is a contraction mediated through α-receptors. Inhibitory responses through β-receptors can be obtained in specimens collected during the luteal phase. This view was recently challenged by Boling and Blandau,[12] however, who found that isolated rabbit oviductal muscle subjected to the influence of estrogens became increasingly active only when progesterone was administered.

In the present work a significant correlation was observed between norepinephrine content of the middle and external portion of Fallopian tubes and plasma progesterone levels, suggesting that ovum passage through the tube may be concomitant with a significant increase in neurotransmitter concentration. Thus, progesterone dominance brings about changes in norepinephrine concentration that may be involved in the acceleration of egg transport during the luteal phase. It should be stressed, however, that even though steroid effects may be mediated in part by changes in the autonomic innervation of the oviduct, sex steroids probably exert influences on oviductal function which are not mediated through adrenergic innervation.

In the uterus, the concentration of norepinephrine did not change as a function of the stage of the ovarian cycle (Table II). In contrast, uterine epinephrine levels decreased by 67 per cent during the luteal phase. Essentially similar results were reported by Cha and associates.[13] These findings on uterine epinephrine content differ remarkably from the absence of changes in tubal epinephrine levels during the menstrual cycle (Table I) as well as from the significant modifications in uterine epinephrine content observed in rats after estradiol treatment.[14, 16] Since tubal and uterine cells, lacking the enzymephenylethanolamine N-methyltransferase (PNMT), are unable to convert norepinephrine into epinephrine, the presence of epinephrine in these cells should come from the blood stream, probably through an active uptake process as suggested by Wurtman and associates.[17]

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Hormonal studies in pregnancy

I. Total unconjugated estrogens in maternal peripheral vein, cord vein, and cord artery serum at delivery

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The concentration of unconjugated total estrogens (primarily estrone, estradiol-17β, and estriol) were measured by rapid radioimmunoassay method in serum samples from maternal peripheral vein, cord vein, and cord artery in normal pregnancies at delivery. The serum concentration of total free estrogens found in cord vein was four to five times higher than in maternal peripheral vein and cord artery. Levels obtained in maternal peripheral vein, cord vein, and cord artery were 47.8 ± 1.8; 140.1 ± 8.57, 31.8 ± 2.15 ng. per millilitre (± standard error of the mean), respectively. These values are compared with those reported by others.

DURING THE LAST two decades a number of investigators have reported levels of free and conjugated estrogens in maternal and cord blood with the use of different techniques.[1–8] The results of these studies have indicated that the placenta is the principal source of the greatly increased amounts of these steroids during pregnancy. The present studies were undertaken to study the levels of total free estrogens in maternal peripheral vein, cord vein, and cord artery blood in normal and abnormal pregnancies, especially pregnancies characterized by intrauterine growth retardation. In this paper we report a rapid radioimmunoassay method for the measurement of total free estrogens and values from normal pregnancies.

Material and methods

Materials. All blood samples were collected in labor or at delivery in normal patients after spontaneous labor. The maternal peripheral vein samples (MV) were drawn in 10 ml. B-D Vacutainers during early labor. The cord artery (CA) and cord vein (CV) samples were drawn either from the clamped cord before delivery of the placenta or from an isolated segment of the cord immediately after fetal delivery. These samples were drawn into dry syringes through 18 gauge needles. The blood was allowed to clot for at least one hour at room temperature then centrifuged and serum removed and stored at −20 degrees C. until analysis.

Ethyl ether, iso-octane, and ethyl acetate were reagent grade obtained from Baker Chemical Co., Phillipsburg, New Jersey. Each freshly opened can of ether was used once and discarded. Iso-octane and ethyl acetate were double distilled and stored in small aliquots. These were used within two to three weeks. Charcoal (Norit A) and dextran T-70 were prepared as a suspension every week. Deionized double-distilled water was used throughout the assay. The counting fluid used was a mixture of Aquasol (New England Nuclear Corp., Boston, Massachusetts) and toluene scintillation grade (1,000 ml. Aquasol and 700 ml. of toluene). Plastic counting vials obtained from New England Nuclear were used. Celite (No. 545), obtained from Fisher Scientific Company, was washed with 6N HCL, distilled water, methanol, and ethyl ether. It was stored at 600° C.

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Table I. Evaluation of the method

Sample	*No. of replicate determinations*	*Mean (ng./ml.)*	*S.E.M.*	*Coefficient of variation (%)*
Cord vein serum	9*	128.5	±0.75	1.56
Cord vein serum	20†	130.0	±1.08	3.70
Pooled cord serum (Control)	13	146.2	±2.5	6.0
Pooled maternal serum in early labor (Control)	12	54.6	±0.82	5.1
Male serum containing 125 ng./ml. estrone + estradiol-17β + estriol (25 ng. + 25 ng. + 75 ng., respectively)	8	120.8	±2.35	4.38
Recovery of added tritiated estriol	100	92.8 ±5.0 (S.D.)	±0.46	4.13

*Determination performed in duplicate at nine different times for intra-assay variation.
†Determination performed in duplicate in one experiment for interassay variation.

All glassware was rinsed with distilled water, methanol, and ethyl ether before use. Kimble disposable tubes (10 × 75 mm.) were used for the assay. Disposable 5 ml. glass pipettes from Kimble or Corning were used for making celite columns. These pipettes were thoroughly washed with detergent, distilled water, methanol, and ethyl ether. Then they were silianized by a one per cent solution of silicad. Eppendorf pipettor was used for addition of charcoal suspension and for the addition of tritiated steroid into assay tubes.

Nonradioactive steroids were obtained from Steraloids Inc., Pawling, New York, and were checked for purity. Standard solutions were prepared in buffer. Radioactive estriol-2,4,6,7-^{3}H (^{3}H-E3) (specific activity 110 c. per millimole), estradiol-17β-2,4,6,7-^{3}H (specific activity 114 c. per millimole), and estrone-2,4,6,7-^{3}H (^{3}H-E_1) (specific activity 110 c. per millimole) were obtained from New England Nuclear Corp., Boston, Massachusetts, and were stored in solvents suggested by the supplier at four degrees C. The purity was checked every three months by paper and thin-layer chromatography. These labeled steroids were purified every three months by water/ether partition followed by celite column chromatography. The purified triatiated steroids were stored at four degrees C. in ethanol solution. Periodically, an aliquot was dried and dissolved in phosphate buffer to obtain a final concentration of 12,000 c.p.m. in 0.1 ml. for the assay and 1,000 c.p.m. in 0.1 ml. for the recovery.

Assay buffer consisted of 0.1M phosphate buffer containing 1 Gm. per liter of sodium azide, 9 Gm. per liter of sodium chloride, and 1 Gm. per liter of gelatin. The pH was 7.0 before adding gelatin. This buffer was used in the assay to dilute samples, to make standard solutions, and to dilute radioactive steroids.

The antiserum to estriol-3, 16α, 17β-trihemisuccinate-HSA (S-310 No. 5) was purchased from Dr. G. E. Abraham.* The cross-reaction of this antiserum with other steroids has been reported by Tulchinsky and Abraham[9] and was checked by us in our laboratory. This antiserum cross-reacts with all three classical estrogens (estriol 100 per cent, estradiol-17β 140 per cent, estrone 140 per cent). It also shows slight cross-reaction (ten to 20 per cent) with some other estrogen metabolites. The antiserum was diluted according to the instructions provided by Dr. Abraham and stored in 0.1 ml. aliquots at −50 degrees C. Final dilution was stored at four degrees C. and used for three to four weeks. The final dilution was such that 0.1 ml. of diluted antiserum bound 50 to 55 per cent of 10,000 c.p.m. (20 pg.) of ^{3}H-estriol.

Rapid method. The serum sample of 20 to 100 μl with 1,000 c.p.m. of ^{3}H-estriol, made up to 1 ml. by deionized glass double-distilled water, was extracted twice with 5 ml. of ethyl ether. The combined ether extracts were evaporated under nitrogen to dryness. The dried extract was dissolved in 1 ml. of assay buffer. An aliquot of 0.5 ml. was used to calculate recovery. Duplicate aliquots of 20, 50, 100, or 200 μl, depending on the concentration of estrogens expected, was used for radioimmunoassay.

The radioimmunoassay method followed is essentially the same as that reported by Tulchinsky and Abraham[9] for estriol. The standard curve was constructed in triplicate with 25 to 400 pg. of estriol. There was no significant variation in the curve from one experiment to another. All samples were counted in a Packard Liquid Scintillation Counter (Model 3320), with an efficiency for ^{3}H of 30 per cent. Samples were normally counted for 20 minutes or 20,000 c.p.m. This gives a counting error of less than one per cent.

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Table II

A. Results

Subject	*Serum*	E_1	E_2	E_3	*Total estrogens,* $E_1 + E_2 + E_3$	*Total estrogen as measured by the rapid method*
1	MV	14.5	38.0	14.9	67.4	64
	CV	27.0	11.0	134.5	172.5	215
	CA	9.5	2.6	41.4	53.5	51.2
2	MV	12.2	19.1	6.3	37.6	45.0
	CV	28.6	8.1	102.6	139.3	125.4
	CA	5.7	3.1	16.6	25.4	29.0
3	MV	4.3	13.3	10.5	28.1	37.0
	CV	16.8	7.6	144.7	169.1	135
	CA	2.4	1.8	26.2	30.4	25
4	MV	17.3	38.0	15.1	70.4	80
	CV	26.6	10.6	164.7	201.9	200
	CA	3.5	1.5	23.2	28.2	32
5	MV	6.9	25.7	14.5	47.2	50
	CV	11.0	7.0	82.1	100.2	100
	CA	2.0	1.6	20.8	24.4	23
6	CV	13.0	7.1	82.2	102.3	115
	CA	2.8	1.9	34.8	39.5	29.6
7	MV	7.9	18.4	18.5	45	50
	CV	37.9	10.7	247.4	296	300.6
	CA	2.7	1.1	19.8	23.6	19.4
8	MV	5.5	21.3	10.76	37.56	35.5
	CV	32.8	7.2	135.2	175.2	168.4
	CA	28.5	7.5	101.2	137.2	148.3

B. Summary

Subjects	*Source of sample*	E_2	E_2	E_3	*Total*	*Total estrogen as measured by the rapid method*
7	MV	9.8 ± 1.8	24.8 ± 3.6	12.9 ± 1.5	47.5	51.6
8	CV	24.2 ± 3.4	8.7 ± 1.1	136.7 ± 19.0	169.6	169.9
7	CA	4.1 ± 1.0	1.9 ± 0.26	26.1 ± 3.3	32.1	29.8

All values are nanograms per milliliter ± S.E.M.

E_1 = Estrone; E_2 = estradiol-17β; E_3 = estriol; MV = maternal vein; CV = cord vein; CA = cord artery.

Table III. Unconjugated total estrogens in maternal vein, cord vein, and cord artery serum at delivery

Source of serum sample	*Number of samples*	*Range (ng./ml.)*	*Mean ± S.E.M. (ng./ml.)*
Maternal vein	49	26-70	47.8 ± 1.8
Cord vein	35	80-250	140.1 ± 8.57
Cord artery	30	17-58	31.8 ± 2.15

With each set of assays four control samples were used. These were: (1) deionized glass double-distilled water stored at four degrees C. in small aliquots, the same used for diluting the serum samples; (2) pooled maternal, pooled cord vein serum, and male serum containing 125 ng. per milliliter of estrogens (estrone, 25 ng.; estradiol-17β, 25 ng.; and estriol, 75 ng.). Water sample served as a check on the method blank. Lately, instead of male serum we use ether-extracted pooled pregnancy serum containing 125 ng. per milliliter estrogens. This also serves as plasma blank.

Chromatographic method. In order to demonstrate the validity of this rapid method we have analyzed in parallel eight serum samples after fractionation of estrone, estradiol-17β, and estriol with the use of celite column chromatography for the separation of these steroids. Estriol antiserum was used.

The serum sample of 50 to 100 μl with 1,000 c.p.m. of estrone and 1,000 c.p.m. of estradiol-17β added made up to 1 ml. by deionized glass double-distilled water was extracted twice with 5 ml. of ethyl ether. The combined ether extracts were evaporated under nitrogen to dryness. Packing of celite column and chromatography was performed as reported by Abraham and associates.[10] The combined ether extract was transferred in three aliquots of 0.3 ml. each with iso-octane on to the celite columns. Elution was first with 3.5 ml. of iso-octane, then with 5 ml. of 15 per cent

Table IV. Findings of other investigators compared with those of the present study

Investigators	*Sample*	*No. of samples*	*Mean (ng./ml.)*			*Total**
			Estrone	*Estradiol-17β*	*Estriol*	
Touchstone and Murawec[2]	Maternal		14.2	8.0	3.6	25.8
	Cord (pooled)		13.0	6.0	69.0	88.0
Adlercreutz and Luukkainen[7]	Maternal	3	11.1	19.0	6.5	36.6
	Cord (pooled)	5	24.3	7.4	137	168.7
Tulchinsky[5]	Maternal	5	5.6 ± 1.12	17.8 ± 3.2	11.8 ± 2.1	30.9
	Cord vein	5	11.0 ± 1.0	6.4 ± 0.72	66.6 ± 20.2	84.0
	Cord artery	5	5.9 ± 1.48	2.7 ± 0.67	32.8 ± 14.0	41.4
Effer et al.[6]	Maternal	11			2.21 ± 0.24	
	Cord vein	16			95.0 ± 19.0	
	Cord artery	16			30.5 ± 9.0	
Wilkinson et al.[12]	Cord	29			139.7	
Simmer et al.[8]	Maternal	10	4.9 ± 0.5	18.1 ± 1.9	11.3 ± 1.6	34.3
	Cord vein	10	22.6 ± 3.0	15.9 ± 1.8	132 ± 16.2	170.5
	Cord artery	10	16.7 ± 1.9	14.6 ± 2.4	109.2 ± 14.1	140.5
Okada et al.[11]	Maternal	6	8.25	25.3	13.8	47.35
Shutt et al.[13]	Maternal	17	10	20	12	42
	Cord vein	6	8.2	7.7	96	108.9
	Cord artery	6	2.5	1.8	19	23.3
Present study	Maternal	49				47.8
	Cord vein	35				140.1
	Cord artery	30				31.8

*Sum of estrone, estradiol-17β, and estriol; not assayed as total estrogens except for the present study.

ethyl acetate in iso-octane (this fraction contains estrone), then with 5 ml. of 30 per cent ethyl acetate in iso-octane to recover estradiol-17β. The tubes were dried under nitrogen, and 1 ml. of assay buffer was added, and then kept at 40 degrees C. for 15 to 20 minutes to dissolve the steroids. An aliquot of 0.5 ml. was used to calculate recovery. Duplicate aliquots of 50, 100, or 200 μl were used for radioimmunoassay.

The radioimmunoassay procedure was the same as that in the rapid method with the exception that standard curves were constructed with the use of a standard solution of estrone and estradiol-17β and tritiated estrone and estradiol-17β solutions were used respectively for the assay instead of estriol. (We found that use of tritiated estriol did not change the values appreciably.)

For the estimation of estriol, the method followed was essentially the same as that reported by Tulchinsky and Abraham.[9]

Results and comment

Recovery. In the rapid method the mean recoveries of labeled estrone, estradiol-17β, and estriol were not significantly different. We have therefore used tritiated estriol as internal standard. The recovery was always found to be over 90 per cent. The mean recovery of 100 samples was found to 92.8 per cent ± 5 per cent (S.D.). In the method involving celite column chromatography the recovery varied from 65 to 90, with a mean recovery for estrone of 77.5 ± 3.8 (S.D.), estradiol-17β, 78.0 ± 5.8 (S.D.), and estriol, 77.8 ± 4.5 (S.D.). All serum values were corrected for procedural losses.

Sensitivity. The sensitivity of the standard curve was 10 pg. for estradiol-17β and 20 pg. for estrone and estriol. Water blanks and celite column blanks were always zero (undetectable). The sensitivity of the method was 0.5 ng. per milliliter.

Precision and accuracy. The method was evaluated for its precision and accuracy and was found to be satisfactory (Table I). The within- and between-assay variance was also determined. The coefficiency of variation was less than five per cent. The interassay variation was 3.7 per cent, while the intra-assay variation was only 1.56 per cent. Three control samples, that is, pooled maternal serum, pooled cord vein, and male serum containing 125 ng. per milliliter estrogens (25 ng. estrone, 25 ng. estradiol-17β, 75 ng. estriol) were used with each experiment. The value of these samples was previously established by repeated analysis in duplicates. This is shown in Table I. In any experiment in which the value of any of these controls was significantly different than the mean, the results were discarded.

Specificity. The specificity of the antiserum was checked by cross-reaction studies with various steroids and was found to be of the same order as that reported by Tulchinsky and Abraham.[9] The cross-reactions of progesterone, dehydroepiandrosterone, testosterone, pregnenolone, and 16α OH-dehydroepiandrosterone

were particularly checked in the rapid method. Addition of up to 5 ng. of these steroids did not change the zero value in the assay. In serum from cord vein values up to 1,500 ng. per milliliter for progesterone have been reported. With the use of the sensitivity and dilution factor it represents only 3 to 4 ng. per tube in our assay. It is therefore not likely to interfere in the rapid method. Other steroids are present in much smaller amounts.

This was further proved by the values obtained by the fractionation of serum for estrone, estradiol-17β, and estriol with the use of celite chromatography. The sum total of the values of estrone, estradiol-17β, and estriol was comparable to the total value obtained by the rapid method, suggesting that other steroids present did not interfere in this rapid method (Table II).

With the use of this rapid method unconjugated total estrogens were measured in maternal peripheral vein serum during labor and in cord vein and cord artery serum from pregnancies classified as clinically normal. The results are reported in Table III. The values for maternal vein, cord artery, and cord vein compare favorably with other reported values (Table IV). The cord artery levels of unconjugated estrone, estradiol-17β, and estriol are very low compared to the levels reported by Simmer and co-workers,[8] while they are similar to those reported by others.[5, 13] Cord vein levels are in agreement with their values. The levels in cord vein were four to five times higher than maternal peripheral vein and cord artery, as expected. Patients with very high levels of estriol in cord artery serum have been found (Table II, Subject 8). Such large amounts of unconjugated estrogens in cord artery possibly reflect the failure of conjugation by the fetus. In another patient total unconjugated estrogen values over 300 ng. per milliliter have been seen in cord vein (Subject 7).

It is not possible to directly compare the total estrogen results with those of others because to our knowledge there is no previous report of the concentration of total free estrogens in maternal peripheral vein, cord vein, and cord artery serums. Most investigators have either reported values for estriol only or for estrone, estradiol-17β, and estriol separately (Table IV).

Further studies are in progress to assess these and other parameters (estrogen precursors) in abnormal pregnancies and attempt to elucidate significant patterns of variations. The total estrogen values obtained by this method compare favorably to those obtained following chromatographic purification of the extract. The method is accurate and faster. It is possible to complete 24 to 36 samples in two working days or 12 samples in one working day by use of this method. We have recently begun to study the feasibility of using this method during the second and third trimester of pregnancy.

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Comparative effects of hyperosmolar urea administered by intra-amniotic, intravenous, and intraperitoneal routes in rhesus monkeys

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Hypertonic urea solutions (58 per cent weight/volume) were injected rapidly into ten anesthetized rhesus monkeys at a dose of approximately 2 Gm. per kilogram by the intravenous, intraperitoneal, or intra-amniotic routes. An additional monkey received an intra-amniotic dose of 12.5 Gm. per kilogram. A tracer dose of ^{14}C-urea was included for measuring absorption and elimination. The following parameters were monitored before and for four hours after urea injection: arterial and cerebrospinal fluid pressures, heart and respiration rates, urine flow, and urea clearance. Serum electolytes, hematocrit and white count, and cumulative urea excretion were measured for one week. Monkeys were observed for three to six months after injection. At doses up to approximately twice the human dose (on a body weight basis) there were no urea-related deaths and no serious side effects noted in any of the experiments. These results support the suggestion that urea is a relatively safe hyperosmolar agent for inducing midtrimester abortions, especially with regard to inadvertent systemic injection.

INTRA-AMNIOTIC HYPEROSMOLAR urea has been reported to be an effective and relatively safe agent for the induction of midtrimester abortion.[1–6] Its chief advantage over the more commonly used hyperosmolar saline is the elimination of the risk of hypernatremia. The modest and transient rise in serum urea that occurs after intra-amniotic urea does not appear to be associated with adverse effects.[6] However, the inadvertent intravenous or intraperitoneal injection of large doses of urea might be expected to exert considerable toxicity. In order to evaluate the seriousness of this risk, we have studied the effects of hyperosmolar urea by the intravenous, intraperitoneal, and intra-amniotic routes in female rhesus monkeys.

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Material and methods

Adult female rhesus (*Macaca mulatta*) monkeys were obtained from the Johns Hopkins Primate Colony. In some experiments pregnant animals were used. Duration of gestation was usually known to within two days. Atropine (0.04 mg. per kilogram and phencyclidine (0.5 mg. per kilogram; Sernylan, Parke, Davis and Co.) were administered preanesthetically, and anesthesia was induced and maintained with 0.5 to 0.8 per cent halothane (Fluothane, Ayerst Labs.) and 50 per cent nitrous oxide via an endotracheal tube. A Foley catheter was inserted into the bladder for continuous collection of urine. Intravenous infusion of five per cent dextrose in 0.45 per cent saline was maintained at a rate of 0.3 to 0.5 ml. per minute with an infusion pump. With the maintenance of sterile conditions, femoral artery pressure was measured directly with saline-filled polyethylene tubing which

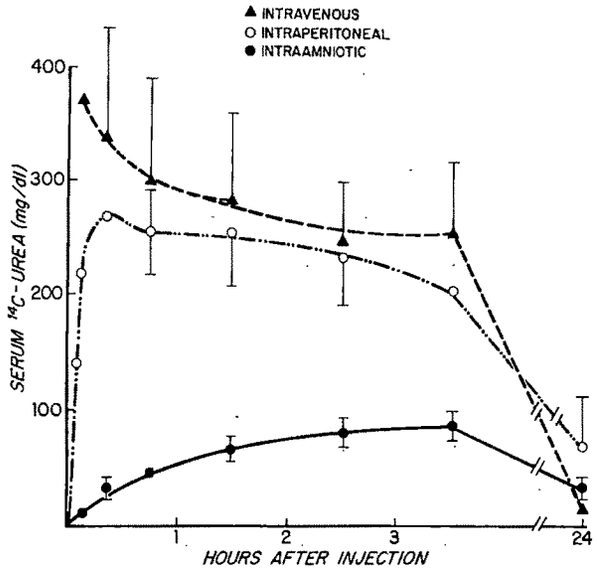


Fig. 1. Serum concentration profile of ^{14}C-urea in rhesus monkeys after rapid injection of approximately 2 Gm. per kilogram as a hyperosmolar (58 per cent weight/volume) solution by three routes. *Points* are means of two or three values and *bars* indicate ±S.E.M.

was connected to a Statham P-23 De pressure transducer. Polyethylene tubing was also inserted into the subarachnoid space by lumbar puncture and connected to a similar pressure transducer for measurement of cerebrospinal fluid (CSF) pressure. A strain gauge was firmly attached to the chest at a position found to give maximal response during respirations. All transducers were connected to a Brush-Gould six-channel polygraph for continuous recording.

Urea was prepared for injection by adding 40 ml. of five per cent dextrose in water to 40 Gm. of sterile lyophilyzed urea (Urevert, Travenol Labs.). This produces a nearly saturated solution of urea having an approximate concentration of 58 per cent weight/volume). ^{14}C-urea (4.5 mc. per millimole; New England Nuclear Corp.) was added to the nonradioactive urea solution so that the injected dose contained approximately 25 μc of radioactivity. The entire dose of urea was injected over three and one-half minutes. Intravenous injections were made into a superficial leg vein. Intraperitoneal injections were administered through a polyethylene catheter which was placed into the peritoneal cavity through a small abdominal incision. Intra-amniotic injections were made through a polyethylene catheter which was threaded through a 16 gauge Tuohy needle inserted transabdominally. Prior to intra-amniotic injection amniotic fluid was withdrawn maximally. In all experiments, immediately after the urea injection was completed, oxytocin (Pitocin, Parke, Davis and Co.) was added to the dextrose/saline infusion at a concentration calculated to deliver 24 mU. per minute which is equivalent on a body weight basis to that used in human abortions.[6]

Arterial blood and amniotic fluid were sampled at the times indicated in Fig. 1. Urine collection intervals bracketed the blood sampling times. Serum, urine, and amniotic fluid were radioassayed by liquid scintillation counting and results calculated as milligrams of urea per milliliter.

Renal clearance of ^{14}C-urea was calculated from the urine flow rate (U), urinary ^{14}C-urea concentration (C), and serum ^{14}C-urea concentration at the midpoint of the urine collection period (S) by the equation: clearance = U × C/S.

Four hours after urea injection, physiologic monitoring was terminated, all catheters removed, incisions sutured, and the monkey permitted to recover from anesthesia. The animal remained in a cage designed for collection of total voided urine for seven postoperative days. Urine was collected and radioassayed on Days 1, 4, and 7.

Venous blood was obtained prior to urea injection and one, four, and seven days afterward for determination of hematocrit, total white cells, sodium, potassium, chloride, bicarbonate, glucose, and urea nitrogen. All hematologic and serum electrolyte determinations were performed by the Division of Clinical Laboratories of the Johns Hopkins Hospital.

Results

Survival. The details of dose and route of urea administration, duration of pregnancy, and outcome of the eleven monkeys studied are given in Table I. Only one urea-related death occurred in a monkey (No. 8) which had been given an intra-amniotic dose equivalent to approximately ten times the usual human dose on a body weight basis. Death occurred 20 hours after the injection and the serum urea nitrogen shortly after death was approximately 1,400 mg. per deciliter. The data from this monkey were not included with the other results. Monkey No. 4 died approximately 24 hours after intraperitoneal injection of urea. The presence of a large amount of blood in the cage and autopsy findings suggested that death was caused by hemorrhage secondary to incomplete abortion. Monkey No. 1 had to be euthanized because sterile procedure was not employed. All surviving animals appeared normal and healthy at three to six months' follow-up evaluation.

Urea serum levels. Fig. 1 shows the mean ± standard error concentrations of ^{14}C-urea in maternal serum during the first 24 hours. The rapid absorption

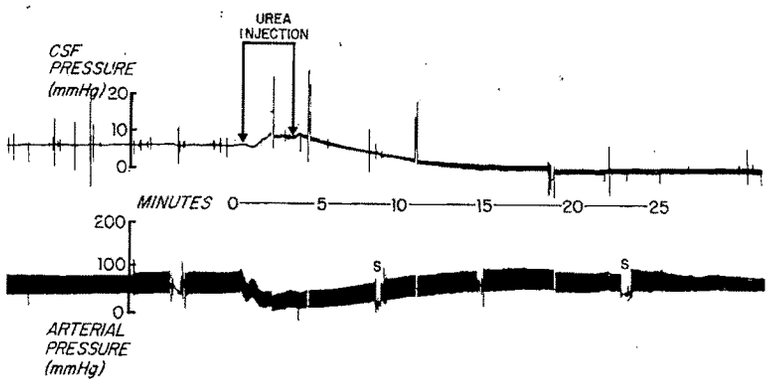


Fig. 2. Arterial and cerebrospinal fluid pressure responses in an anesthetized rhesus monkey (No. 3) to intravenous injection of hyperosmolar urea. *S* = Sampling time.

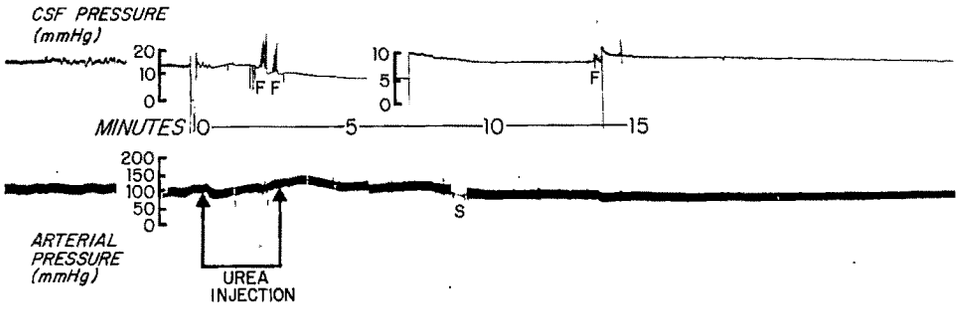


Fig. 3. Arterial and cerebrospinal fluid pressure responses in an anesthetized rhesus monkey (No. 7) to intraperitoneal injection of hyperosmolar urea. *F* = Catheter flush.

of intraperitoneal urea is apparent in that the serum concentration peaked within 30 minutes and nearly reached that measured after intravenous injection. For this reason, the results of other measurement in the intravenous and intraperitoneal groups were combined for comparison to those obtained in the intra-amniotic group.

The average serum concentration of intravenous urea was 375 mg. per deciliter four minutes after the end of the three and one-half minute injection. It then decreased exponentially with a half-time of five to six hours (determined graphically on a semilog plot). A similar rate of elimination was estimated for urea after intraperitoneal and intra-amniotic administration. The apparent volume of distribution of urea after intravenous injection was estimated by extrapolation of a semilog plot to the theoretic concentration at zero time. The injected dose divided by the zero time concentration of urea gave an apparent volume of distribution of 70 to 85 per cent of body weight slightly higher than expected if the distribution was equal to total body water (approximately 60 to 65 per cent of body weight).

Arterial and cerebrospinal fluid pressures. Intravenous injection of hyperosmolar urea caused a rapid but evanescent fall in arterial pressures followed by a slow rise to above the preinjection level and a return to it within 30 minutes (Fig. 2). These pressure changes were not accompanied by any marked or consistent change in heart rate. Intraperitoneal urea injection caused a modest increase in both systolic and diastolic pressures reaching a peak in about five minutes (one and one-half minutes after completion of the urea injection) and then returning to preinjection levels in another 10 minutes (Fig 3). CSF pressure rose transiently during the intravenous injection and then fell slowly to 5 to 15 mm. Hg below the preinjection level at two hours with a slower rise toward base line thereafter. A similar fall in CSF pressure occurred after intraperitoneal injection of urea. No significant or consistent changes occurred in arterial pressure after intra-amniotic injection. However, CSF pressure decreased slowly by an average of 5 mm. Hg at 60 minutes, returning to preinjection levels by four hours.

Respiration. The rate of spontaneous breathing did

Table I. Summary of experiments

Monkey	*Route*	*Urea dose (Gm./Kg.)*	*Pregnancy duration (days)*	*Outcome*
1	IV	1.8	154	Euthanized four hours after urea injection
2	IV	2.4	NP	Survived
3	IV	1.7	NP	Survived
4	IP	1.8	133	Died; hemorrhage during abortion
5	IP	1.6	132	Aborted and survived
6	IP	1.8	NP	Survived
7	IP	1.7	NP	Survived
8	IA	12.5	135	Died 20 hours after injection
9	IA	2.2	75	Survived and resorbed fetus
10	IA	2.3	69	Survived and resorbed fetus
11	IA	2.5	85	Survived and resorbed fetus

IV = intravenous; IP = intraperitoneal; IA = intra-amniotic; NP = nonpregnant adult female.

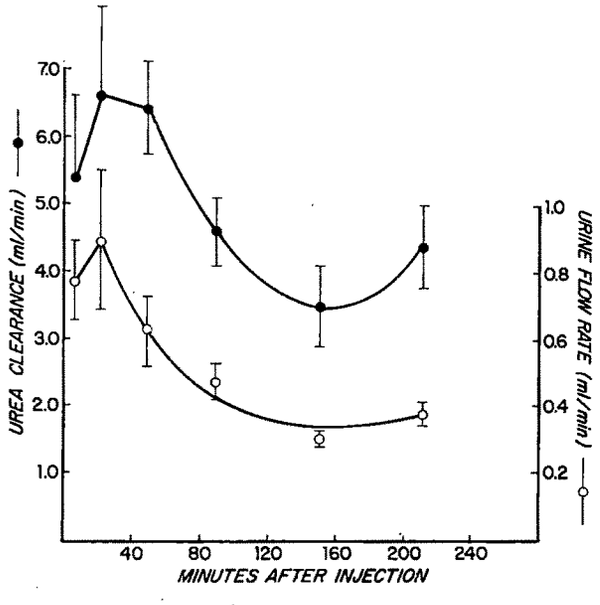


Fig. 4. Urea clearance and urine flow in anesthetized rhesus monkeys after intravenous or intraperitoneal (combined data) injection of hyperosmolar urea (2 Gm. per kilogram). Values are means ± S.E.M. of three to six animals. Oxytocin (24 mU. per minute) in 5 per cent dextrose/0.45 per cent saline intravenous infusion started a few minutes after urea injection and maintained at a rate of 0.3 to 0.5 ml. per minute throughout.

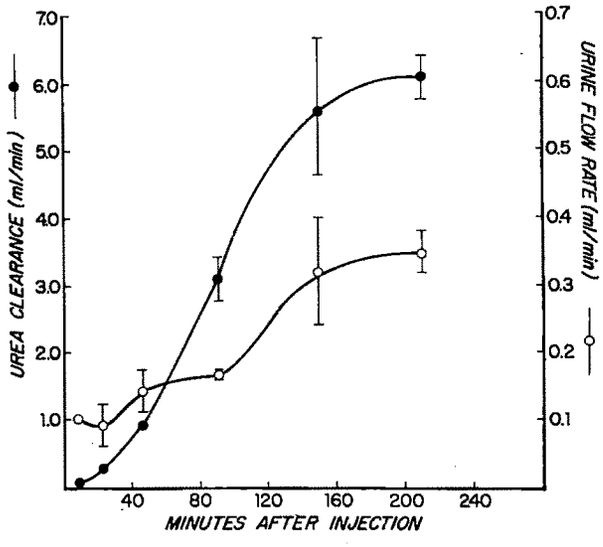


Fig. 5. Urea clearance and urine flow in anesthetized pregnant rhesus monkeys after intra-amniotic injection of hyperosmolar urea. See Fig. 4 for other conditions. Values are mean ± S.E.M. of three animals.

not show discernable changes in any of the experiments.

Urine flow rate and urea clearance. Intravenous and intraperitoneal injection of urea caused an initial diuresis to a maximum at approximately 20 minutes after the start of the injection and then tapering off to a flow rate approximating the rate of the dextrose/saline infusion (0.3 to 0.5 ml. per minute) (Fig. 4). As expected, urea clearance by the kidney showed a parallel increase during the period of diuresis.

After intra-amniotic injection, urine flow and urea clearance steadily increased to a plateau level at about two and one-half hours (Fig. 5). Urea clearance was significantly higher three to four hours after intra-amniotic injection (6.1 ± 0.3 ml. per minute) than after intravenous/intraperitoneal injection (4.4 ± 0.6 ml. per minute). However, it should be noted that all of the intra-amniotic monkeys in this group were pregnant, whereas only three of seven of the intravenous/intraperitoneal group were pregnant.

Serum electrolytes. Fig. 6 shows the standard serum electrolytes before and one, four, and seven days after urea injection. The data for intravenous and intraperitoneal monkeys have been combined for comparison with those of the intra-amniotic animals. The only notably persistent change was a decrease in serum

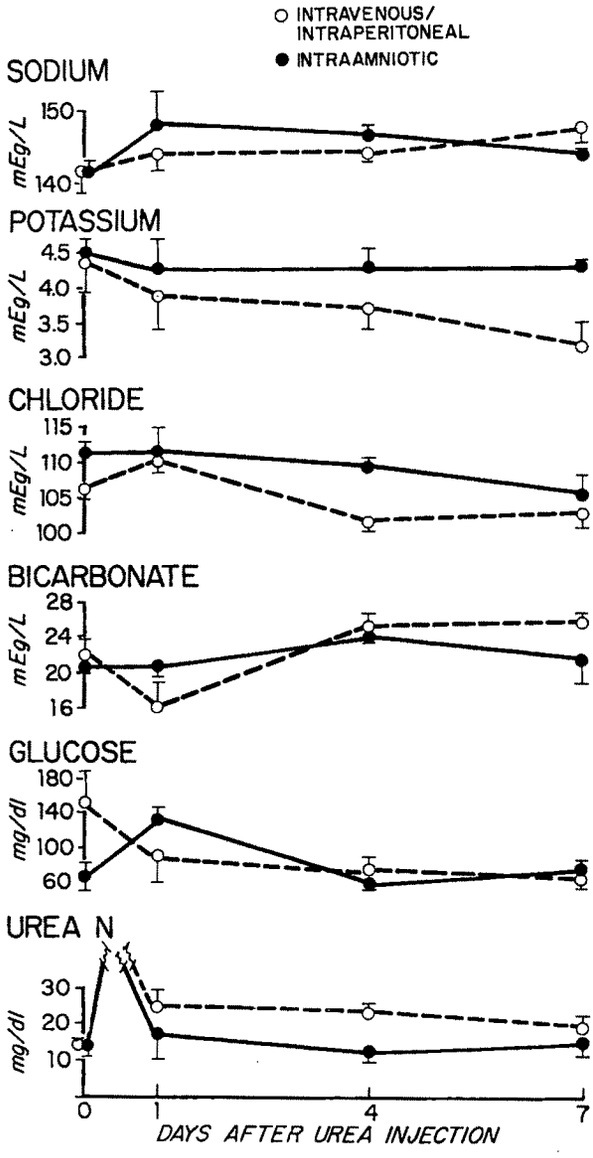


Fig. 6. Serum electrolytes in rhesus monkeys before and after injection of hyperosmolar urea. Values for intravenously and intraperitoneally injected animals have been combined. Points are means ± S.E.M. of three to six animals.

potassium in the monkeys which received systemic injections of urea. Serum chloride and bicarbonate also tended to be lower in this group, suggesting renal loss of these electrolytes during an osmotic diuresis.

Serum urea nitrogen (SUN) levels decreased rapidly during the first day and were within or near normal limits in all animals. Measurement of ^{14}C-urea in urine showed a cumulative 24 hour excretion of 60 to 85 per cent of the dose for the intravenous group, 70 to 110 per cent in the intraperitoneal group, and 35 to 60 per cent in the intra-amniotic group. Urea excretion after the first day was small except in the intra-amniotic

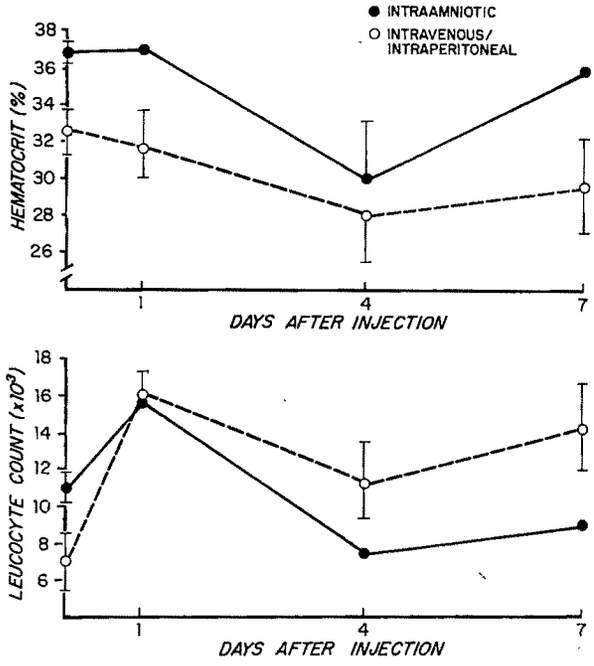


Fig. 7. Hematologic measurements in rhesus monkeys before and after injection of hyperosmolar urea. Values for intravenously and intraperitoneally injected animals have been combined. *Points* are means of two to five animals. *Bars* indicate ± S.E.M.

group in which an additional 15 to 20 per cent was recovered between Days 1 and 4.

Hematology. Because of surgical blood loss and blood withdrawal for various measurements (35 to 50 ml.), the hematocrit decreased moderately in all animals between Days 1 and 4 but was increasing toward the preinjection level within one week (Fig. 7). Marked hemolysis and hematuria was observed for one to two hours after intravenous injection, however, the hematocrits in this group were not different from those of the intraperitoneal injected animals. The total white cell count increased by 50 to 200 per cent within 24 hours in all monkeys, regardless of the route of injection of urea.

This leukocytosis was transient in the intra-amniotic group but persisted for at least seven days after intravenous/intraperitoneal injection of urea.

Comment

Hypernatremia inadvertently produced by hypertonic saline when being used as a midtrimester abortifacient has been reported to be the cause of serious and possibly fatal complications including hemolysis, hypotension, cardiac arrest, apnea, bradycardia, and arrhythmias.[7, 8]

Because of these potential hazards associated with

saline, other hyperosmolar agents such as urea have been investigated for efficacy and safety as midtrimester abortifacients.[9] Although the use of urea has been increasing over the past few years, we are not aware of any serious complications or deaths associated with elevated urea concentration. Indeed, up to 2 Gm. of urea per kilogram of body weight has been administered over a two-hour period to sickle cell patients without serious side effects.[10] However, the rapid elevation of the osmolar concentration of extracellular fluid with urea causes encephalopathy and death in rabbits[11] and marked pulmonary hypertension[12] and systemic hypotension[13] in dogs. Therefore, the safety evaluation of hypertonic urea for use as a midtrimester abortifacient requires an assessment of its toxicity when administered in a manner designed to simulate possible inadvertent intravenous or intraperitoneal injection. Because urea freely diffuses across cell membranes, it would not be expected to produce as much of an osmotic gradient as an equiosmolar dose of a substance such as sodium whose passage across cell membranes is retarded. Moreover, urea is an electrochemically neutral molecule and thus does not directly alter excitable membranes in cardiac and nervous tissue. Thus it is not too surprising that rapid intravenous or intraperitoneal injection of a very large dose (approximately 2 Gm. per kilogram of body weight) of a nearly saturated solution of urea (approximately 10M) did not cause death or persistent physiologic or biochemical changes in monkeys. The transient changes in blood pressure observed during and shortly after urea injection were similar to those reported by other investigators[12, 14] under similar dosage conditions. The observed decrease in cerebrospinal fluid pressure is a well-known effect of intravenously injected urea and has been used clinically in the treatment of excessive intracranial pressure.[15]

The urea effects on renal function are difficult to evaluate because of the antidiuretic effect of coadministered oxytocin. It was felt necessary to administer oxytocin in these studies as it is usually employed as an augmenting agent clinically. It was evident, however, that systemically administered urea caused a diuresis and enhanced urea clearance when compared to intra-amniotic injection.

Marked hemolysis was noted for approximately two hours after intravenous urea injection. Thus it was somewhat surprising to find hypokalemia from one to seven days after injection. Burnett and associates[6] observed in patients a slight rise (0.5 mEq. per liter) in serum potassium 12 hours after intra-amniotic injection of urea, with a return to normal limits within 24 hours. A possible explanation for these observations is that the potassium released from lysed red cells was excreted during the first day during osmotic diuresis. This total body potassium depletion along with the decrease in dietary potassium intake during postoperative anorexia probably resulted in the observed hypokalemia. Changes in renal function may also be related to the marked hemolysis that occurred with intravenous injection.

Mild to moderate leukocytosis has been observed in most patients after intra-amniotic urea injection.[6] The mechanism of this effect is unknown, but it may be associated with the formation of endogenous prostaglandins which are thought to be responsible for many of the effects of hypertonic agents.[16]

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Pharmacokinetics of intra-amniotically administered hyperosmolar urea in rhesus monkeys

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Three rhesus monkeys in midgestation were injected intra-amniotically with hyperosmolar ^{14}C-urea (58 per cent weight/volume, 2.3 Gm. per kilogram, 25 μc) and the concentration of ^{14}C-urea measured serially for 24 hours in amniotic fluid, maternal serum, and maternal urine. The volume of the amniotic space was measured by isotope dilution of ^{99m}Tc-albumin. The experimental values were fitted to a three-compartment mathematical model with the aid of a digital computer program for nonlinear data. The computer-derived concentration versus time curves were in close agreement with the measured values when the flux of urea was primarily from amniotic fluid to fetal water to maternal water with insignificant direct transport between the amniotic fluid and maternal serum. The half-times for distribution of urea into fetus and mother were approximately 30 minutes and 7 hours, respectively. Thus, although the uptake of urea by the fetus from surrounding amniotic fluid is rapid, the further movement of urea from fetus to mother is relatively slow.

UREA HAS BEEN advocated as a substitute for sodium chloride in midtrimester abortion by the intra-amniotic (IA) hyperosmolar technique.[1] The primary advantage of urea over saline is the relative lack of toxicity associated with inadvertent intravenous or intraperitoneal injection.[2, 3] Although there are numerous reports on the clinical abortifacient efficacy of hyperosmolar urea in relatively large series of patients,[4–7] there have been no detailed studies published on its rate of absorbtion and elimination after intra-amniotic injection in human beings or animals. Burnett and associates[7] measured serum urea nitrogen (SUN) in a series of twelve elective midtrimester abortions with 80 Gm. of hyperosmolar urea. The average peak SUN occurred at four hours after injection and returned to preinjection values at 24 hours.

There is evidence that the site of action of hyperosmolar abortifacients is in the decidual cell layer underlying the chorioamnion.[8] Therefore a major determinant of abortifacient activity may be the product of concentration of solute × hours in amniotic fluid which would be proportional to the solute concentration in the decidual environment. Furthermore, the passage of hyperosmolar solute from amniotic fluid into the fetus presumably results in a loss of cardiac function after a critical fetal osmolarity is exceeded.

This investigation was conducted in order to gain insight into these fundamental pharmacologic aspects of the abortifacient action of hyperosmolar urea. Pregnant rhesus monkeys were utilized as a model for human beings and experiments conducted in a manner designed to simulate the clinical technique most commonly employed. At various times after IA injection of ^{14}C-urea as a hyperosmolar solution, its concentration was measured in amniotic fluid, maternal serum, and urine. These values were fitted to a

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Table I. Urea dosage and amniotic fluid volumes

Variables	*Monkey No.*			
	1	*2*	*3*	*Average*
1. Body weight (Kg.)	5.9	6.4	5.5	5.9
2. Urea dose (Gm./Kg.)	2.3	2.2	2.5	2.3
3. Pregnancy duration (days)	69	75	85	76.3
4. Volume of amniotic fluid (ml.)				
a. Initial (A)*	22	95	93	70
b. After amniocentesis ($A_{resid.}$)†	4	44	18	22
c. After urea injection (t = 0)‡ ($A_{resid.} + V_{inj.}$)	29	69	43	47
d. After urea injection (t = 3.5 hr.)§	45	84	58	62
5. Urea concentration in amniotic fluid at t = 0 (mg./ml.) ‖	200	97	86	128
6. Amniotic urea space (ml.) (V_d [urea])¶	73	154	169	132
7. Apparent fetal water volume (ml.) (F)#	44	85	126	85

*Determined by isotope dilution analysis with ^{99m}Tc-albumin.

†Difference between 4a and volume obtained by amniocentesis.

‡Volume of hyperosmolar urea solution (25 ml.) plus 4b.

§Determined by isotope dilution analysis with ^{99m}Tc-albumin.

‖Determined by extrapolation of semilog plot of amniotic fluid urea concentration versus time to t = 0.

¶Determined by dividing urea dose by values in Line 5.

#Determined by subtracting values in Line 4c from those in Line 6.

three-compartment mathematical model with the use of a digital computer program and the relevant pharmacokinetic parameters obtained. This model was used to predict the urea concentration versus time curve for the fetus.

Material and methods

Three midgestation rhesus monkeys obtained from the Johns Hopkins University Primate Colony had been cohabitated with males for two to 12 days during the middle of a menstrual cycle, and pregnancy confirmed by bimanual palpation of the uterus four weeks after mating. The presumed date of conception was the midpoint of the cohabitation time which was designated Day 1 of gestation. All three animals had at least two previous normal pregnancies. The gestational ages and maternal body weights are given in Table I.

Food was withdrawn the night before the experiment. Atropine sulfate (0.04 mg. per kilogram) and phencyclidine hydrochloride (Sernylan, Parke, Davis and Co.; 0.5 mg. per kilogram) were administered prior to maintenance of anesthesia with halothane (Fluothane, Ayerst Labs.; 0.5 to 0.7 per cent) and nitrous oxide (50 per cent) in oxygen. Venous, arterial, and cerebrospinal fluid catheterizations were performed for physiologic monitoring which is reported elsewhere.[3] A venous infusion of five per cent dextrose in 0.45 per cent saline was maintained at a rate of 0.4 to 0.5 ml. per minute throughout the five hours of anesthesia. A No. 10 Foley catheter was placed in the bladder for continuous urine collection.

Hyperosmolar urea was prepared by dissolving 40 Gm. of sterile lyophylized urea (Urevert, Travenol Labs.) in 40 ml. of five per cent dextrose in water producing a solution of approximately 58 per cent (weight/volume) with respect to urea. Approximately 25 μc of ^{14}C-urea (New England Nuclear Corp., 4.5 mc. per millimole) was added to 30 ml. of this solution and 25 ml. of the resultant mixture (containing 13.8 Gm. of urea) used for IA injections. Doses calculated as grams per kilogram are given in Table I.

Prior to aspiration amniocentesis, the volume of amniotic fluid was estimated by the isotope dilution technique with ^{99m}technetium-labeled albumin obtained from the Johns Hopkins Radiation Health Laboratory. ^{99m}Tc-albumin (2 to 5 μc) was dissolved in 1 ml. of 0.9 per cent saline and injected into the amniotic cavity through a polyethylene catheter (PE-90) which had been threaded through a previously inserted 16 gauge Touhy needle. Thorough mixing was assured by repeated withdrawal and infusion from the syringe and allowing 15 to 30 minutes prior to sampling. Amniotic fluid samples (0.5 ml.) were withdrawn at various times and counted in a Baird Atomic II isomatic gamma counter. The volume of amniotic fluid was estimated as the apparent volume of distribution of the injected ^{99m}Tc-albumin calculated with reference to a known dilution.[9] These results are given in Table I (Line 4a).

Maximal amniotic fluid was withdrawn and the volume noted. Urea injection was made through the polyethylene catheter at a steady rate over 3.5 minutes.

Experimental time was designated zero at the start of the injection. Upon completion of the injection, fluid was repeatedly withdrawn and infused to ensure thorough mixing. Immediately after completion of the urea injection, oxytocin (Pitocin, Parke, Davis and Co.) was added to the intravenous infusion at a concentration calculated to deliver 20 to 40 mU. per minute which is approximately equal on a body weight basis to the human dose utilized for augmentation of urea-induced abortions.[7]

Amniotic fluid (0.5 ml.) and blood (2 ml.) were obtained before and at 7.5, 12.5, 15, 22.5, 45, 90, 150, 210, 480, 720, 960, and 1,440 minutes after injection of urea. Total urine was collected at 0, 15, 30, 60, 120, 180, and 240 minutes by catheter. Catheters were removed and the animals allowed to recover from anesthesia after transfer to cages. Urine collection continued until 24 hours by pan collection. In one experiment (No. 3) the monkey was placed in a restraining chair from four to 24 hours and catheter urine collection continued. Aliquots of amniotic fluid, maternal serum, and urine were radioassayed by liquid scintillation counting (Nuclear Chicago Model 725). Counting efficiency was determined by the channels ratio method and disintegrations per minute converted to milligrams of urea equivalent per milliliter. Cumulative urinary excretion of urea was calculated from summation of concentration times volume per interval.

The apparent volume of distribution of urea based on amniotic fluid concentration was calculated by dividing the dose by the concentration in amniotic fluid extrapolated to zero time (C_0). The extrapolation was performed on a semilog plot of ^{14}C-urea concentration in amniotic fluid versus time by extending the slower linear phase (linear on semilog coordinates) back to time equals zero hours, as shown in Fig. 1. Assuming that IA urea freely and rapidly diffuses into the fetus and distributes with total fetal body water at a faster rate than it distributes into maternal body water, this volume [V_d(urea)] was assumed to be approximately equivalent to the volume of amniotic fluid remaining prior to the urea injection ($A_{resid.}$) plus the volume of urea solution injected ($V_{inj.}$) plus total fetal body water (F); (V_d[urea] = $A_{resid.}$ + $V_{inj.}$ + F). The residual amniotic fluid volume after aspiration ($A_{resid.}$) was obtained by subtracting the volume obtained by aspiration ($V_{asp.}$) from the initial volume measured by ^{99m}Tc-albumin dilution (A). The results of these calculations are given in Table I.

An estimate of the first-order rate constant for transfer of urea from amniotic fluid into fetal water was obtained by the "curve-stripping" or "feathering" technique,[10] as shown in Fig. 1. The dashed line represents the early rapid (alpha) phase of the apparent biphasic decline of urea concentration in amniotic fluid thus obtained. The later slower (beta) phase was assumed to represent transfer of urea from the amniotic-fetal compartment to maternal water, and the half time ($t_{\frac{1}{2}}$) of this decay was used to estimate the first-order rate constant for this process (K = 0.693/$t_{\frac{1}{2}}$). The rate constant for elimination of urea from maternal water was estimated from semilog plots of its concentration in serum versus time after intravenous injection of a similar dose into nonpregnant animals.

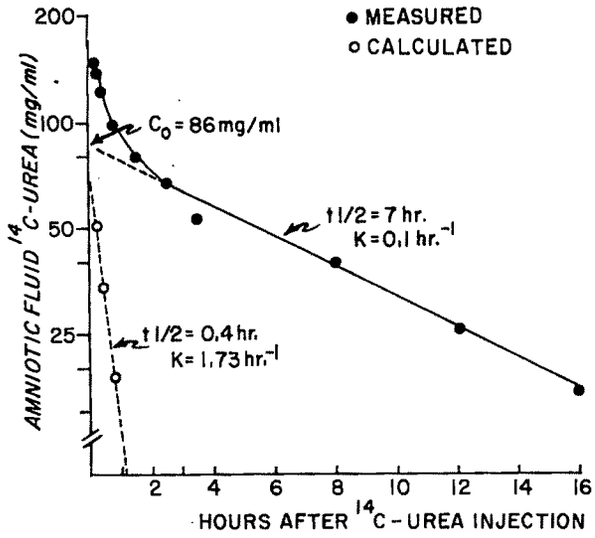


Fig. 1. Semilog plot of ^{14}C-urea elimination from amniotic fluid after intra-amniotic injection of a hyperosmolar solution (58 per cent) in a midgestational rhesus monkey (Experiment No. 3). *Dotted line* (early phase) was obtained by curve-stripping technique.[10] C_0 = apparent initial concentration obtained by back extrapolation of the postdistributive linear phase. Graphically estimated half-times ($t_{\frac{1}{2}}$) used to obtain estimates of the apparent first-order rate constants *(K)* by K = 0.693/$t_{\frac{1}{2}}$.

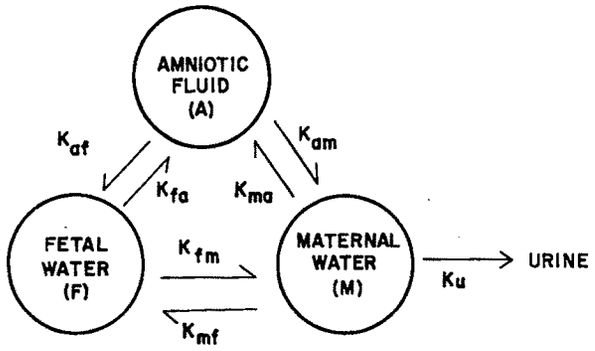


Fig. 2. Compartmental scheme for transfer of urea to fetal and maternal water after intra-amniotic injection.

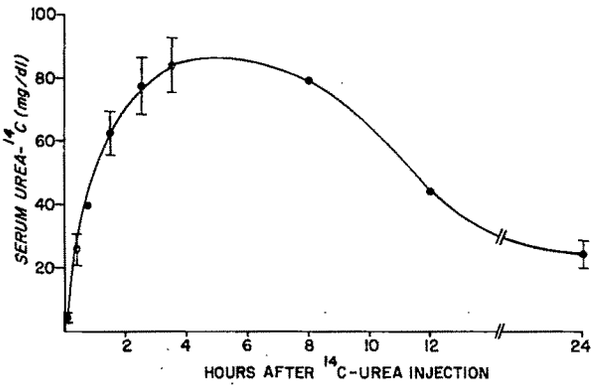


Fig. 3. Maternal serum ^{14}C-urea concentration versus time after intra-amniotic injection of a hyperosmolar solution (58 per cent) in midgestational rhesus monkeys. Points are mean values of two or three animals. Bars indicate standard errors of the mean.

Table II. Pharmacokinetic parameters for three-compartment fit of urea concentration and excretion data

Variables	*Monkey No.*			
	*1**	*2*	*3*	*Average*
Pregnancy duration				
days	69	75	85	76.3
%	42	45	52	46.3
K_{am} (hours^{-1})	0.010	0.036	0.007	0.018
K_{ma} (hours^{-1})	0.069	0.006	0.007	0.027
K_{af} (hours^{-1})	1.153	1.142	1.154	1.150
K_{fa} (hours^{-1})	1.086	1.105	1.086	1.092
K_{fm} (hours^{-1})	0.321	0.312	0.321	0.318
K_{mf} (hours^{-1})	0.067	0.051	0.007	0.042
K_u (hours^{-1})	0.200	0.204	0.198	0.200

*Parameters do not represent a least-squares fit because of incomplete urine collection.

These data are reported elsewhere.[3] That this rate constant represents urinary excretion was evident from its similarity to the rate constant obtained from measurements of urea clearance by the kidneys assuming the volume of distribution of urea to be 70 per cent of adult body weight. Thus it was concluded for the purposes of these calculations that nonrenal maternal losses of injected ^{14}C-urea were insignificant.

The urea concentrations in amniotic fluid and maternal serum and cummulative urinary excretion values along with the estimated first-order rate constants and estimated compartment volumes were used to fit the three-compartment scheme shown in Fig. 2 to the experimental data. An IBM370 and digital computer program* developed to aid in fitting nonlinear functions to data were used in obtaining the set of parameters that describe the model in Fig. 2. The parameters obtained allow a "best fit" of the data based on least-squares criteria.

*We thank Dr. Carl Metzler of the Upjohn Company for providing the computer program and details of its utilization.

Results and comment

The body weights and urea dosage were similar in all three experiments (Table I). Although at a similar gestational age, it is apparent from Table I that there were significant differences in amniotic fluid volumes and apparent fetal volumes in the three monkeys. Consequently, at a fixed urea dose, the initial concentration of urea in amniotic fluid (Table I, Line 5) decreased with advancing fetal size. An attempt was made to maximally aspirate amniotic fluid prior to urea injection in order to have minimal dilution of injected hyperosmolar urea and to simulate the clinical technique. The estimates of fetal water volumes (Table I, Line 7) correspond to those expected based on published combined rhesus fetal and placental weights[11] (103 Gm. at 75 days gestation and 249 Gm. at 100 days) and aggregate tissue water contents of approximately 85 per cent (volume/weight).[12] For example, in Monkey No. 2, gestational age 75 days, 85 per cent of the expected combined fetal (43 Gm.) and placental (60 Gm.) weights is 88 ml., a value close to the 85 ml. calculated. Unfortunately, although strong and regular uterine contractions occurred, no fetal weights were available for confirmation because the treatment resulted in missed abortions followed by fetal resorption.

Urea is a small water-soluble molecule (molecular weight = 60) which crosses biologic membranes with relative ease and distributes into a space presumed to be approximately equal to total body water.[13] In addition, it is reasonable to assume that urea distributes across body membranes by simple diffusion through aqueous channels and that the rate of its equilibration between two compartments (for example, fetal and maternal) is primarily limited by water flow between those compartments.[14]

Furthermore, since the total body pool of endogenous urea (85 mg. per kilogram) and the rate of its production (13 mg. per kilogram per hour)[15] are small relative to the dose and elimination rate of ^{14}C-urea injected in these experiments, for the purpose of analysis of the data, the specific activity of the ^{14}C-urea was assumed to remain constant.

From the declining concentration of ^{99m}Tc-albumin radioactivity in amniotic fluid during the first three and one-half hours after urea injection it would appear that its volume was increasing slowly, presumably because its hypertonicity caused an influx of water along the

osmotic gradient (Table I, Line 4d). It was surprising that although the concentrations of urea and thus tonicity differed considerably in the three experiments, the rate of apparent volume expansion was nearly identical in each at approximately 5 ml. per hour. In one experiment (No. 3) the apparent amniotic fluid volume was determined at 24 hours to be 65 ml., only 7 ml. more than that determined 20 hours earlier. Some of the apparent volume expansion (dilution of ^{99m}Tc radioactivity) may have been the result of incomplete binding of the label to albumin or dissociation caused by hyperosmolar urea. Ultrafiltration of amniotic fluid containing ^{99m}Tc-albumin showed that six to ten per cent of the radioactivity was unbound and could have moved out of the amniotic space. Additionally, a small but measurable fraction of albumin is known to be lost from amniotic fluid within ten minutes after injection.[9] Consequently, it was believed unnecessary to account for the small changes of urea concentration in amniotic fluid which possibly resulted from volume expansion.

The biphasic exponential decay in amniotic fluid urea concentration determined in experiment No. 3 is shown in Fig. 1. The initial phase had a $t_{\frac{1}{2}}$ of 24 minutes and the later phase had a $t_{\frac{1}{2}}$ of seven hours. It is assumed that the initial rapid phase represents passage into the fetal and placental tissues and the slower phase represents transfer to maternal water. The amniotic fluid to fetal transport probably represents direct percutaneous passage as fetal skin has been shown to be freely penetrated by isotopic water in midgestation prior to keratinization.[16] There is also evidence that water exchange between amniotic fluid and maternal serum occurs through the cord.[17] Fetal swallowing of amniotic fluid during midgestation is estimated to be only five to ten per cent of fetal body weight per hour, a process too slow to account for the observed rate.

Fig. 3 shows the maternal serum ^{14}C-urea concentrations for the three monkeys. The time of peak serum level (four to six hours) and the shape of the curve approximates that reported clinically.[7] The values are approximately twice those reported in human beings corresponding to the approximate double dose on a weight basis administered to monkeys.

The concordance between measured and computer-predicted urea concentrations in amniotic fluid and maternal serum determined for experiment No. 3 is shown in Fig. 3. This figure also shows the excellent fit obtained with the cumulative amount of urea excreted in urine. The computer-predicted curve for the fetal urea concentrations is also shown, although no direct measurements were available for comparison. Two important observations can be made from Fig. 3. First,

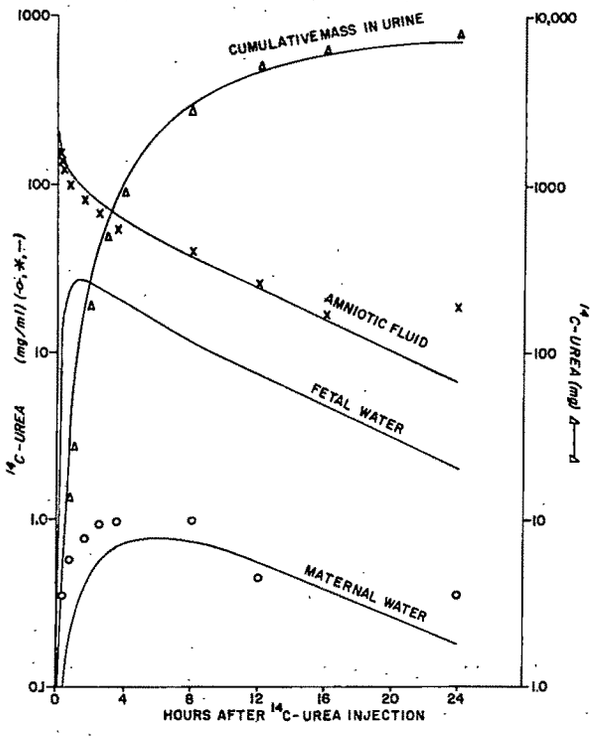


Fig. 4. Computer-derived fits of urea measurements from Experiment No. 3 to the three-compartment model shown in Fig. 2. Ordinates are log scale: *left* for concentrations in amniotic fluid and fetal and maternal water; *right* for cumulative amount in maternal urine. First-order rate constants for best least-squares fit are given in Table II.

the urea concentration in the fetal compartment rises rapidly and peaks at approximately one and one-half hours, corresponding to the rapid initial decline in amniotic fluid urea shown in Fig. 1. Second, although an equilibrium is achieved between amniotic fluid and fetal urea concentration after two hours, the concentration in the fetal compartment remains substantially below that in amniotic fluid even though the apparent rate constant into the fetus is considerably larger than that from the fetal to maternal compartments (Fig. 1 and Table II). The most likely explanation for this observation is that while urea equilibration between amniotic fluid and fetal skin may be rapid, equilibrium of urea within the fetus and placenta is blood flow limited. Blood flow–limited exchange would also explain the even greater differential (approximately 70-fold) existing between urea concentrations in the fetal and maternal compartments.

The computer-derived first-order rate constants for best fitting the measured data to the three-compartment interconnected model (Fig. 2) are given in Table II. Attempts to obtain other best fits by altering initial estimates or weighting the measured values by a factor equal to their reciprocals did not

result in a substantially better fit based on the least-squares determination. An interesting finding is the relative insignificance of direct transfer of urea from amniotic fluid to the maternal compartment (K_{am}). The probability that a molecule of urea in the maternal compartment arrived from the amniotic fluid via the fetal compartment is more than 30 times greater than the probability of its arrival directly. This means that only about three per cent of the urea enters the maternal compartment directly from amniotic fluid. The finding of low urea flux between amniotic fluid and maternal serum is in agreement with the reports of studies of tracer doses of ^{14}C-urea in pregnant rhesus monkeys[15] and in vitro with human chorion laeve.[18] Since most of the urea that appears in maternal serum has previously been transported to the fetus and then across the placenta, a relatively slow rise and delayed peak is seen in the time course of concentration in the maternal serum (Fig. 3). As expected, the rate constant for transfer of urea from amniotic fluid to the fetal compartment (K_{af}) was independent of the concentration in amniotic fluid (*see* Table I).

With the use of a mathematical model involving the amniotic fluid, fetal water, maternal water, and maternal urine, experimentally measured concentrations of ^{14}C-urea after intra-amniotic injection of a hyperosmolar urea solution were fitted with the aid of a digital computer program (Fig. 4). The first-order rate constants derived from this model demonstrate that the transport of urea from amniotic fluid to the maternal circulation is retarded because the solute must first pass through the fetus and placenta. This finding explains the surprisingly modest elevation in maternal serum urea observed after intra-amniotic injection of large doses of hyperosmolar urea for midtrimester abortion.

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Patients at high risk for low-birth-weight delivery

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A retrospective analysis of 8,839 singleton deliveries was undertaken to determine the relationships of maternal age, maternal hemoglobin concentration, and past obstetric performance to the incidence of low-birth-weight (LBC) delivery. Patients under 19 years of age and patients with hemoglobin values less than 9.0 Gm. per 100 ml. were found to have significantly higher incidences of LBW deliveries. In addition, the incidence of LBW delivery was found to vary in proportion to the number of previous LBW deliveries and inversely with the number of previous term deliveries.

IT IS GENERALLY accepted that premature delivery is the most common obstetric complication associated with perinatal death, accounting for the majority of all such fatalities occurring at this time.[1, 2] In addition, surviving infants of low birth weight due to prematurity or fetal growth retardation have high incidences of neurologic, intellectual, and emotional deficits.[3, 4] The incidence of prematurity in the United States is generally quoted as being between 7 and 14 per cent. If one assumes an annual birth rate of three million and an incidence of 8.5 per cent prematurity, then approximately 250,000 premature infants are born annually in the United States alone.[5] Although this serious complication of pregnancy occurs with alarming frequency, an acceptable cause is identifiable in only 40 per cent of the cases. The maternal complications that most frequently predispose to premature labor are hypertensive cardiovascular disease, abruptio placentae, and placenta previa. In these and other circumstances of maternal and fetal jeopardy, premature delivery may be iatrogenic in that termination of pregnancy is required to protect the lives of the mother and her baby. If one adds multiple pregnancies and congenital anomalies of the fetus to this list, approximately 40 per cent of premature deliveries are accountable. The remaining 60 per cent of premature labors are without apparent explanation.[1]

In those deliveries characterized by a birth weight below 2,500 grams, premature labor is usually accepted as the most common cause, although fetal growth retardation is said to account for one third.[6] The differentiation of these etiologic factors is not always possible, but clearly LBW delivery is a major factor associated with obstetric wastage. For purposes of improved management and clinical investigation, it is desirable to identify those maternity patients who are at high risk. The objectives of this study were to determine the relationship of the following three factors to the incidence of LBW delivery: (1) maternal age, (2) maternal hemoglobin concentration, and (3) past obstetric performance, particularly previous premature delivery.

Methods

The obstetric population utilized for this retrospective study was the 8,839 black patients delivered at the University of Maryland between 1955 and 1960. Those patients with multiple pregnancies and those who had had therapeutic terminations of their pregnancies were excluded from the analyses.

Maternal age was determined on the basis of information obtained upon the first prenatal visit. For purposes of this study, the hemoglobin concentration assigned to each patient was the lowest value determined antenatally. The past obstetric performance was derived primarily from the history. In an effort to test the cumulative effects of past obstetric events, the

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Table I. Maternal age and incidence of LBW delivery

Age (yr.)	*Deliveries*	*LBW delivery (%)*	
<15	90	18.9	20.3
15-19	2,659	20.4	
20-24	2,756	16.4	15.8
25-29	1,543	13.6	
30-34	1,055	17.1	
35-39	545	16.0	
40-44	177	16.9	
45 and over	14	14.3	
Total	8,839	17.2	

Table II. Maternal hemoglobin concentration and incidence of LBW delivery

Anemia	*Deliveries*	*LBW delivery (%)*	
5-5.9	4	25	27.9
6-6.9	10	20	
7-7.9	28	39	
8-8.9	101	26	
9-9.9	666	17	16.8
10-10.9	1,723	16	
11 + >	6,126	17	
Total (with Hgb recorded)	8,648	17	
Sickle cell	20	20	
Megaloblastic	3	67	

patients were subdivided into groups according to the number of previous LBW deliveries and the number of previous mature deliveries.

Because gestational ages could not be accurately ascertained in all patients, it was decided to define LBW delivery as one yielding an infant with a birth weight of 400 to 2,499 grams. Although the majority of such infants are premature, a significant proportion are mature, growth-retarded infants. On the basis of the data available, it was not possible to differentiate these two groups of LBW infants. Those with birth weights of 2,500 grams or more were designated mature, or term. Although a small proportion of these infants were probably premature, this differentiation also was not possible from the available data.

Results

Of the total 8,839 singleton pregnancies analyzed in this study, 1,475 resulted in the delivery of infants weighing between 400 and 2,499 grams. This represents an over-all incidence of LBW delivery of 16.7 per cent in this black population.

Table I demonstrates the incidence of LBW delivery according to maternal age. When the mother was 19 years or younger, the over-all incidence was 20.3 per cent. This was significantly higher than the 15.8 per cent incidence of LBW delivery noted when the mother's age was 20 years or greater ($p < .001$).

Table III. The incidence of LBW delivery according to past obstetric performance

Previous term deliveries	*Previous LBW deliveries*			
	0	*1*	*2*	*3 + >*
0	19.3% (435/2,253)*	35.3% (77/218)	54.1% (40/74)	59.7% (37/62)
1	15.1% (217/1,439)	28.4% (62/218)	36.4% (24/66)	45.2% (28/62)
2	11.0% (112/1,018)	21.6% (42/194)	27.0% (16/59)	46.2% (18/39)
3	9.2% (59/642)	16.9% (26/154)	39.7% (23/58)	50.0% (16/32)
4 + >	9.8% (154/1,571)	14.7% (61/416)	20.6% (22/107)	23.1% (6/26)

	Previous abortions		
	0	*1*	*2 + >*
0	19.3% (435/2,253)	31.8% (35/110)	45.0% (9/20)

*Number of LBW deliveries/total deliveries.

Table II indicates the incidence of LBW delivery in relation to the lowest recorded antenatal hemoglobin value. The incidence of LBW delivery was found to be significantly greater in those patients with a hemoglobin value less than 9.0 grams (27.9 per cent) when compared to those with a hemoglobin concentration of 9 grams or greater (16.8 per cent) ($p < .001$). The 20 per cent incidence observed in patients with sickle-cell disease is in good agreement with that reported in other series.[7] The 67 per cent incidence observed in patients with megaloblastic anemia should be accepted with caution, since only three such cases were identified.

Table III demonstrates the frequency of LBW delivery in relation to the number of past term and past LBW deliveries. The groups are subdivided according to the number of previous term deliveries (0, 1, 2, 3, and 4 or greater); previous LBW deliveries (0, 1, 2, and 3 or more); and previous spontaneous abortions (0, 1, and 2 or more). A striking rise in the rate of LBW delivery is observed with increased numbers of past LBW deliveries. This relationship is consistently present in that increasing numbers of previous LBW deliveries were always associated with increasing incidences of subsequent LBW delivery. Within those groups of patients subdivided according to the number of previous term pregnancies, a three- to fivefold increase in the incidence of LBW delivery was noted between patients that had not had previous LBW

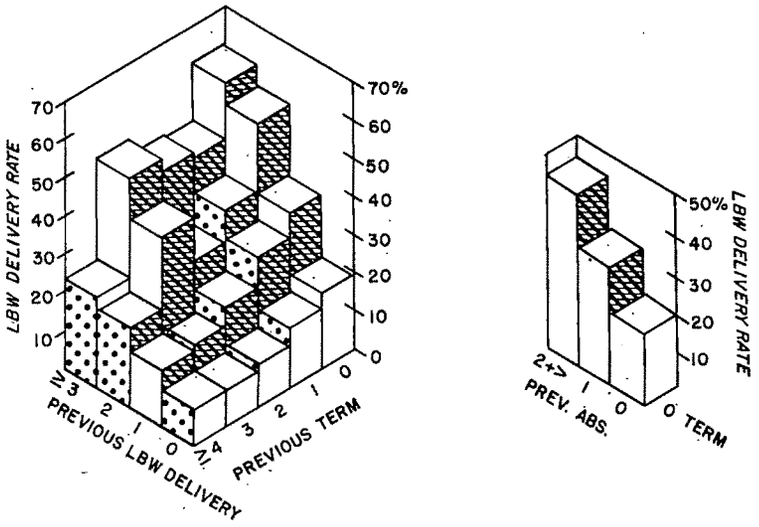


Fig. 1. In the left-hand graph, LBW delivery rates are represented on the vertical axis, according to the number of previous LBW deliveries and previous term deliveries. In the right hand graph, the LBW delivery rates are depicted according to the number of previous spontaneous abortions in nulliparous patients.

deliveries and those that had had three or more. For example, in the group of patients who had had three previous term pregnancies, the observed incidence of LBW delivery was 9.2 per cent among those who had not experienced previous LBW delivery, as opposed to 50 per cent among those who had had three or more previous LBW deliveries ($p < .001$).

The converse relationship between previous term pregnancies and subsequent LBW delivery is also remarkable in that increasing numbers of previous term pregnancies were usually associated with decreasing incidences of subsequent LBW delivery. Within groups subdivided according to the number of previous LBW deliveries, a 50 to 62 per cent reduction in the incidence of LBW delivery was noted between those patients who had not had previous term pregnancies and those who had had four or more.

The association between previous spontaneous abortions and subsequent LBW delivery among nulliparous patients is also shown in Table III. A striking increase in LBW delivery was noted with increasing numbers of previous miscarriages (19 to 45 per cent; $p < .001$).

The relationships shown in Table III are depicted in graphic form in Fig. 1. Those columns with cross-hatching represent LBW delivery rates significantly greater than observed in the corresponding patient group without previous LBW delivery. Those columns with dots represent LBW delivery rates significantly less than the corresponding group without previous term pregnancies. In the portion of the graph demonstrating the relationship between past miscarriages and LBW delivery rates, the columns with cross-hatching represent LBW delivery rates significantly in excess of those observed in patients without previous miscarriage.

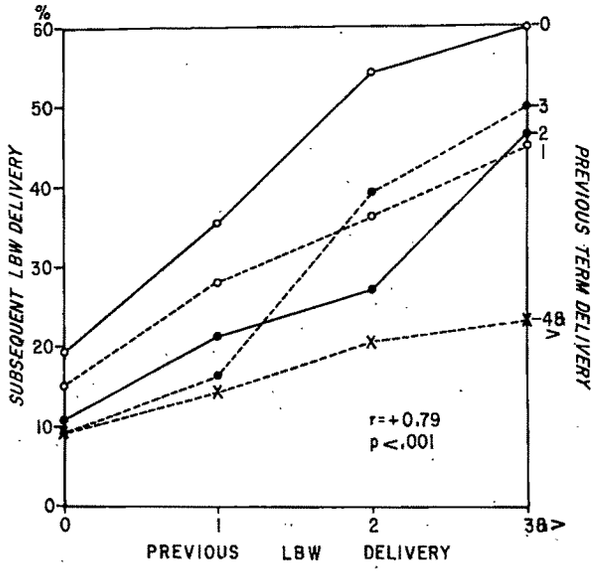


Fig. 2. This graph demonstrates the relationship between varying numbers of previous LBW deliveries and subsequent LBW delivery rates. The rates for patient groups with the same number of previous term pregnancies are joined by lines.

In Fig. 2, the incidences of LBW delivery in the study pregnancies have been plotted against the incidences of previous LBW deliveries. A positive correlation

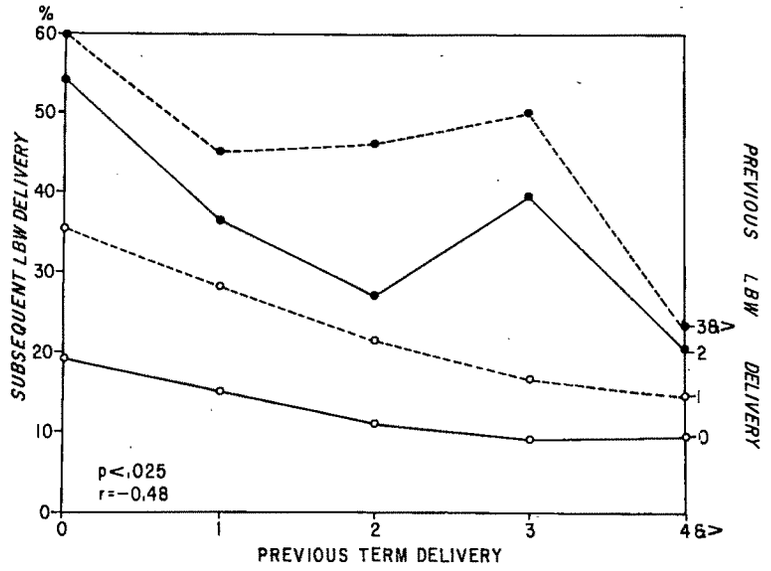


Fig. 3. This graph illustrates the relationship between varying numbers of previous term deliveries and subsequent LBW delivery rates. LBW delivery rates for groups with the same number of previous LBW deliveries are joined by lines.

coefficient was found to be present when the values for all term pregnancies were considered ($r = +0.79$; $p < 0.001$). The values for those patient groups with the same number of previous term pregnancies have been connected by identity lines for purposes of further analysis as discussed below.

In Fig. 3, the incidences of LBW delivery in the study pregnancies have been plotted against the number of previous term deliveries. In this case, a significant negative correlation coefficient was found to be present when the values for all groups were considered ($r = -0.48$; $p < 0.025$). The points for those patient groups with the same number of previous LBW deliveries have been joined by lines for purposes of further analysis as given below.

Comment

Although it would be more meaningful to distinguish those LBW deliveries associated with prematurity from those associated with intrauterine growth retardation, this was not possible in the present study. The LBW babies studied in these analyses undoubtedly include a number of infants whose birth weights were small for gestational age and who did not represent truly premature infants. The relationships demonstrated probably apply to true prematurity, however, since they have been reported in other studies where prematurity was defined according to gestational age.[8]

The increased incidence of LBW delivery noted in the young patients of this study is a common observation reported by many previous investigators.[9, 10] No satisfactory explanation for this association is provided by the present study, although a low socioeconomic status has been suggested as a common factor.[10] The mechanisms have not been documented by which a low socioeconomic status might predispose to premature delivery.

The association between anemia and prematurity has also been noted previously.[11] In a study of teenage pregnancies, Israel and Woutersz[12] concluded that anemia was more common in teenage mothers and that age rather than anemia per se was more likely to account for the increased incidence of prematurity. In the present study, no correction for age nor for socioeconomic status was made in the analysis of the relationship between hemoglobin values and prematurity. Griswald and Cavanagh,[13] in comparing indigent patients with term deliveries and indigent patients with premature deliveries, found the incidence of anemia was only 6 per cent higher in the latter population.

The impact of previous LBW delivery and term delivery. The repetitive tendency of LBW deliveries has been reported by many observers.[8–10] To the authors' knowledge, however, previous studies have not shown the cumulative risk associated with increasing numbers of prior LBW deliveries. Nor has the cumulative benefit of prior term pregnancies been demonstrated.

The mechanism by which LBW delivery leads to successive LBW deliveries remains to be determined. On the basis of the lines representing the values for different term pregnancy groups (Fig. 2), the relationships appear to be linear with the slopes varying inversely with respect to the number of previous term pregnancies. Increasing numbers of past LBW deliveries appear to have an additive effect upon the incidence of subsequent LBW delivery.

The effect of previous term pregnancies on the incidence of subsequent LBW delivery, as depicted in Fig. 3, is opposite to that of previous LBW delivery. In general, increasing numbers of previous mature deliveries are associated with decreasing incidences of LBW deliveries. Thus, previous LBW delivery and previous maturity appear to have cancelling effects, with respect to the incidence of subsequent LBW delivery. Whereas the effect of previous low birth weight delivery appears to be additive, however, that of previous mature delivery does not appear to be simply subtractive. In Fig. 3, the curves connecting points for those patients with less than two previous LBW deliveries appear to be exponential, approaching an irreducible minimum of 10 to 15 per cent incidence. If the two curves representing patients with two or more previous LBW deliveries are extrapolated through the points representing 0, 1, and 4 or more previous term deliveries, they also appear to be exponential, approaching a minimum value of 10 to 15 per cent. The fact that the remaining three values are well above the extrapolated lines might be due to the influence of previous LBW deliveries overriding the opposing influence of previous term pregnancies. It might also be attributed to the fact that these points represent relatively small patient groups. In any event, the exponential feature of these lines suggests that increasing numbers of previous mature deliveries have a divisional, rather than subtractive, effect on the rate of subsequent LBW deliveries. The apparent minimum frequency of 10 to 15 per cent may represent the random occurrence rate in this population.

Since these data are derived from the late 1950's, some question might be raised as to their relevance with respect to present-day obstetric outcome. On the basis of these data, patients who have had two or more previous LBW deliveries, might be expected to experience a subsequent LBW delivery rate of 30 to 60 per cent. In fact, in a prospective study performed during 1971, 1972, and 1973, patients who had had two or more previous premature deliveries were found to experience an over-all incidence of prematurity of about 40 per cent.[14] Furthermore, factors known to be associated with recurrent prematurity (cervical incompentency, fundal anomalies, placenta previa, and abruptio placentae) were infrequently observed in this patient population. Further investigations of this unique high-risk population may give important information on the etiology of idiopathic prematurity.

The impact of previous miscarriages. Although Ross and Schneider[10] suggested that previous spontaneous abortions predispose to subsequent premature delivery, Griswald and Cavanagh[13] were unable to demonstrate such a relationship. The discrepancy between the latter study and our own might be related to the fact that the other authors did not correct for the number of previous term pregnancies. In the present study, evaluation of the effect of previous abortions was limited to nulliparous patients. The incidence of LBW delivery may relate to previous abortions and previous term pregnancies, as it does to previous LBW deliveries and previous term pregnancies. Under these circumstances, it would be necessary to study the effect of abortion within groups subdivided according to the number of previous mature deliveries.

The 45 per cent incidence of LBW delivery observed in nulliparous patients who have had two or more previous miscarriages is a striking increase of this complication. It is of interest that an increased incidence of premature delivery has recently been reported also as a late sequel to induced abortions.[15] It remains to be determined whether the mechanisms are similar that account for the increased incidences of prematurity following spontaneous and induced abortions.

In summary, this retrospective study demonstrated that, within the patient population analyzed, the very young patients and those patients with anemia were at an increased risk for delivery of LBW infants. In addition, those patients who had had previous LBW deliveries or miscarriages were noted to be at an increased risk of subsequent LBW delivery. The risk of LBW delivery appeared to vary directly with the number of previous LBW deliveries, and inversely with the number of previous term deliveries.

The identification of patients at an increased risk for LBW delivery should aid in their clinical management, since the opportunity is thus presented to focus on interconceptional and early prenatal care. In addition, the identification of a specific high-risk patient population can be of assistance in clinical studies and investigations pertaining to the etiology and management of LBW pregnancies.

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Amniotic fluid osmolality in pregnancies complicated by diabetes

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Amniotic fluid osmolality (AF Osm) was studied in 247 patients whose pregnancies were complicated by maternal diabetes. Significant increases in mean AF Osm accompanied pre-existing fetal as well as subsequent perinatal death. Abnormal increases in AF Osm were associated with a significant increase in risk of perinatal compromise with cumulative morbidity and mortality rates of 54 per cent. These data suggest that serial evaluation of AF Osm enables detection of a "higher risk" group of diabetic pregnancies in which perinatal death is more than doubled.

THE RELATION BETWEEN amniotic fluid osmolality (AF Osm) and fetal compromise has been a recent topic of clinical concern in perinatal medicine.[1-7] Definitive data concerning this relation, particularly in the pregnancy complicated by diabetes, are sparse.[7] The present report attempts to provide preliminary data in this regard.

Material and methods

During a three-year period, 1970 to 1972, a total of 485 amniotic fluid samples were obtained by transabdominal amniocentesis from 247 patients whose pregnancies were complicated by diabetes. Samples were obtained for the most part during prenatal visits to a high-risk perinatal clinic; samples were not obtained during labor. In 119 patients, single AF samples were available, while in 128 serial samples were obtained. Duration of gestation was estimated from menstrual history and confirmed in many cases with fetal sonar measurements as well as by Dubowitz[8] examination of the newborn infant. Criteria for diagnosis of gestational diabetes were similar to those of O'Sullivan and associates,[9] the diagnosis of Class A diabetes being made if whole blood glucose exceeded 110 mg. per cent (fasting), 170 mg. per cent (one hour), 120 mg. per cent (two hours), or 110 mg. per cent (three hours) following a 100 Gm. oral glucose load (GTT). Class AD diabetes (gestational diabetes with carbohydrate decompensation) was diagnosed if whole blood glucose exceeded 120 mg. per cent (fasting) or 180 mg. per cent (two hours) after GTT or if whole blood glucose exceeded 300 mg. per cent at any time. Severity of insulin-requiring diabetes (Classes B to D) was estimated with the criteria of White and co-workers.[10]

Electronic fetal monitoring was performed in many patients and both one- and five-minute Apgar scores were determined at delivery for all patients. Those with electronic evidence of type II fetal bradycardia,[11] predelivery passage of meconium, and/or one- or five-minute Apgar scores of ≤6 were considered to have perinatal asphyxia. Following delivery, most of these infants were cared for in the University High-Risk Nursery where they were screened for biochemical evidence of *hypoglycemia* (serum "true sugar" ≤30 mg. per cent if <37 weeks' gestation or ≤40 mg. per

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Table I. Clinical features of 247 study pregnancies complicated by diabetes

Clinical parameter	*Result*
Race (black/white)	163/84
Age	
Years	15-45
Mean ± S.D.	26.5 ± 7.3
Class of diabetes	
A	167 (67.6%)
AD	33 (13.4%)
B	21 (8.5%)
C	16 (6.5%)
D	10 (4.0%)
Death	
Fetal	15 (60.7/1,000)
Neonatal	6 (25.9/1,000)
Perinatal	21 (85.0/1,000)
Morbidity	77 (31.2%)
Macrosomia	49 (19.8%)
IGR	26 (10.5%)
Vascular disease	95 (38.5%)

cent if ≥37 weeks' gestation.[12] Perinatal morbidity was considered present in any infant with evidence of perinatal asphyxia and/or hypoglycemia.

Intrauterine growth retardation (IGR) was diagnosed when birth weight was ≤10 percentile of that expected for gestation, race, and sex; macrosomia was considered present when birth weight was ≥90 percentile of that expected.[13] Diastolic blood pressure ≥90 mm. Hg on two successive examinations and/or proteinuria led to the diagnosis of renovascular disease. As distinction between acute or subacute maternal pre-eclampsia and persistent or pre-existing vascular compromise (hypertensive cardiovascular or renovascular disease) was often not possible in our population, no attempt was made to distinguish between these forms of maternal vascular disease.

Amniotic fluid osmolality was determined by freezing point depression.* Samples were free from overt meconium and/or blood, centrifuged, refrigerated, and analyzed in duplicate or triplicate within 48 hours of receipt. Data were subjected to chi square or Student's t test.

Death rates were calculated as:

$$\text{Fetal death rate} = \frac{\text{fetal deaths}}{\text{total births}} \times 1{,}000.$$

$$\text{Neonatal death rate} = \frac{\text{neonatal deaths}}{\text{total live births}} \times 1{,}000.$$

$$\text{Perinatal death rate} = \frac{\text{neonatal deaths + fetal deaths}}{\text{total births}} \times 1{,}000.$$

*Advanced Instruments Osmometer Model 31LS.

Table II. Relation of severity of diabetes to perinatal mortality, morbidity (asphyxia and hypoglycemia), altered fetal growth (intrauterine growth retardation or macrosomia) and maternal vascular disease

	Class of diabetes		
Clinical parameter	*A (167)*	*AD (33)*	*B, C, D (47)*
Perinatal mortality	24.0 (4)*	151.5 (5)*†	255.3 (12)†
Morbidity	29% (48)	33% (11)	38% (18)
Macrosomia	17% (28)	27% (9)	26% (12)
IGR	10% (17)	6% (2)	15% (7)
Vascular disease	37% (62)	55% (18)‡	32% (15)‡

*$p < 0.005$.
†$p < 0.001$.
‡$p < 0.05$.

Table III. Relation of maternal vascular disease to perinatal death, morbidity, and altered fetal growth in the 167 study pregnancies

	Vascular Disease	
Clinical parameter	*Present (95)*	*Not detected (152)*
Perinatal death	114.6 (11)	65.4 (10)
Fetal macrosomia	26% (25)*	16% (24)*
Fetal growth retardation	12.5% (12)	9% (14)
Perinatal morbidity	31% (30)	31% (47)

Number of patients indicated in parenthesis.
*$p = <0.025$.

Results

Clinical features of the study pregnancies. Table I summarizes certain clinical features of the 247 pregnancies studied. The study subjects were predominantly black (66 per cent) and the pregnancies for the most part were complicated by gestational diabetes (81 per cent). One fifth of the women were teenagers (49/247), nearly one third in their 30's or 40's (73/247), and half were in their 20's (120/247). Maternal vascular compromise was common. Fetal, neonatal and total perinatal mortality rates in the study group were nearly three times those of our population as a whole during the same years (perinatal mortality rate 85.0 in study group/30.6 in total population 1970 to 1972). In nearly one third of the perinatal patients, asphyxia and/or hypoglycemia was documented (77/247) and one in five was large for gestational age (49/247). Fetal growth retardation was no more common in these patients (26/247 = 10.5 per cent) than that in the general population (ten per cent by definition[13]).

Table II summarizes the effect that severity of maternal diabetes had on the frequency of certain clinical problems. Excess perinatal death was confined

Table IV. Relation of amniotic fluid osmolality to certain clinical findings in the study pregnancies

Patient group	*Number of fluids*	*Osmolality* (mOsm./Kg.)*	*p*	*Gestational age* (wk.)*	*p*
Perinatal morbidity					
Yes	167	258.4 ± 11.2	<0.02	36.1 ± 3.5	N.S.
No	277	255.8 ± 11.0		36.7 ± 3.2	
Fetal death					
Yes	28	272.2 ± 16.7	<0.001	33.3 ± 5.4	<0.001
No	277	255.8 ± 11.0		36.7 ± 3.2	
Neonatal death					
Yes	13	264.9 ± 10.6	<0.005	34.3 ± 3.1	<0.01
No	277	255.8 ± 11.0		36.7 ± 3.2	
Altered fetal growth					
Macrosomia	87	256.0 ± 12.6	N.S.	37.0 ± 3.2	<0.05
IGR	50	261.1 ± 11.8	N.S.	35.7 ± 4.2	N.S.
Normal fetal growth	110	257.8 ± 11.7		36.0 ± 3.9	
Severity of disease					
Class A	303	255.2 ± 11.1		37.1 ± 3.2	
Class AD	84	260.7 ± 8.5	<0.001	34.6 ± 3.7	<0.001
Class B-D	98	263.3 ± 14.4	<0.001	34.4 ± 4.6	<0.001
Vascular disease					
Yes	188	258.9 ± 11.5	N.S.	36.1 ± 3.3	N.S.
No	297	257.2 ± 12.2		36.2 ± 4.1	

Gestational ages at time of sampling are provided for each group.
*Mean ± one standard deviation.

to patients of Class AD or greater severity; the rate of 24.0/1,000 in patients with Class A diabetes was similar to that of our general population, but the rate of 212.5/1,000 for those of AD, B, C, and D severity was significantly higher (4/167 versus 17/80; $p < 0.005$). The incidence of perinatal morbidity (asphyxia and/or hypoglycemia) tended to increase with severity of maternal diabetes, but these differences were not statistically significant. Combined mortality and morbidity rates (52 of 167 in Class A = 31 per cent; 16 of 33 in Class AD = 48 per cent; 30 of 47 in Class B-D = 64 per cent) also increased with severity of diabetes; these risks were significantly lower in Class A than in Class AD, B, C, D patients ($\chi^2 = 15.706$; $p < 0.001$). Fetal macrosomia, frequent in all groups, tended to be more common in Classes AD, B, C, and D (21 of 80 = 26 per cent) than in the Class A patients (28 of 167 = 17 per cent), but this difference was not significant. The incidence of fetal growth retardation in Class B, C, and D patients (seven of 47 = 15 per cent) was higher than that in Class A or AD (19 of 200 = nine per cent), but this trend also was not statistically significant. Vascular disease, common in all groups, was more frequent in Class AD patients (18 of 33 = 55 per cent) than in Class A (62 of 167 = 37 per cent) or Classes B to D patients (15 of 47 = 32 per cent); this difference was significant only for the later comparison ($p < 0.05$). The impact of maternal vascular disease on the perinatal infant is further analyzed in Table III. A nearly twofold significant increase in fetal macrosomia in patients with vascular disease was observed (25 of 95 versus 24 of 152; $p = < 0.05$). The incidence of perinatal morbidity was identical in both groups.

In Table IV AF osmolality is related to certain clinical complications in the study pregnancies. Mean osmolality was significantly higher in amniotic fluids obtained from pregnancies with ultimate perinatal death or morbidity, fetal growth retardation, or more severe maternal diabetes than that from those without complications. Fluids from these compromised groups, however, were generally obtained earlier in gestation. As AF osmolality is inversely related to gestational age,[4] further analysis of these data, balanced for gestational age, is presented in Fig. 1. From the data in Fig. 1, it is clearly apparent that, with correction for gestational immaturity, increased AF osmolality consistently correlates only with perinatal death or more severe (Classes B, C, and D) maternal diabetes. In point of fact, increased AF osmolality correlates only with perinatal death; with exclusion of figures from Class B, C, and D patients with perinatal death, mean AF osmolality in the more severe diabetic patients is virtually identical to that in the Class A group (at < 32 weeks, 264 versus 262 mOsm. per kilogram; at 33 to 36 weeks, 257 versus 256 mOsm. per kilogram; at 37 to 41 weeks, 257 versus 254 mOsm. per kilogram, respectively).

Serial AF osmolality measurements were obtained in

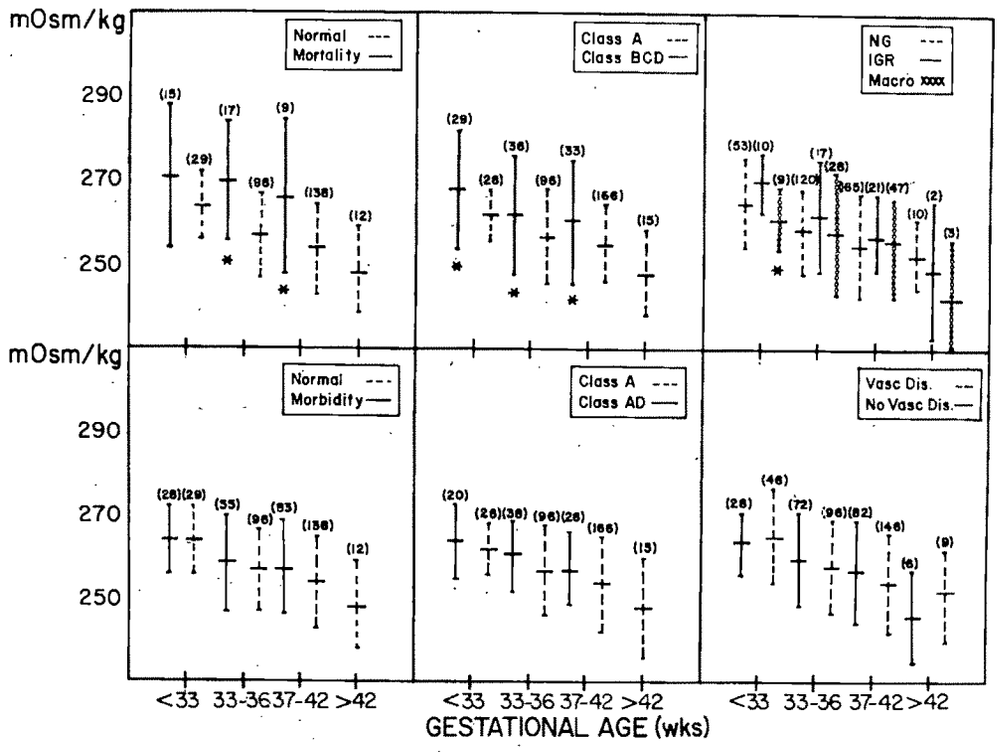


Fig. 1. Amniotic fluid (AF) osmolality (mean ± one standard deviation) at grouped gestational ages in diabetic pregnancies. Number of AF samples in parentheses. Asterisk indicates statistically significant difference between means ($p < 0.05$).

128 patients. Stable or declining values, expected with advancing gestation, were observed in 65 pregnancies; perinatal death occurred in four (six per cent) and morbidity was present in another 19 (a cumulative 35 per cent). The relation of serial AF osmolality to perinatal outcome is summarized in Fig. 2. Perinatal death in the 63 pregnancies with abnormal, serially rising AF osmolality was doubled (9/63, 14 per cent) and morbidity was present in another 25 (a cumulative 54 per cent; $p < 0.05$). Individual AF osmolality values for all 13 perinatal deaths in this series are provided in Fig. 3 in order to demonstrate that consistently altered amniotic fluid osmolality did not invariably precede or predict subsequent perinatal death.

Comment

Amniotic fluid osmolality (AF Osm), primarily dependent on sodium and accompanying anion concentrations in water,[2] declines linearly during late gestation.[7] A temporal relation to improved fetal tubular resorption of sodium[14–16] and increased gut resorption of sodium accompanying increased swallowing movements[17–18] as well as a return of AF Osm toward maternal serum levels following fetal death[1] or life-threatening fetal manipulative surgery[19] suggest an active if not central role for the fetus in determining the ultimate hypoosmolality of the amniotic fluid.[7]

In view of these observations, it is not surprising that several investigators have observed increased AF Osm to accompany a variety of perinatal compromises. Initial work from our laboratory concerned 79 pregnancies complicated by a variety of problems, including Rh sensitization, diabetes, toxemia, and/or hydramnios. Mean AF Osm was significantly increased with prior or subsequent perinatal death as well as in situations of more severe fetal hemolytic disease with survival.[2] Serial AF Osm evaluations were obtained in 48 patients with Rh sensitization; abnormal serial increases in 23 patients were accompanied by a 40 per cent perinatal mortality rate (ten times that of the 25 patients with normal serial declines) and cumulative morbidity and death occurred in 96 per cent (44 per cent in those with decreasing osmolality). While the most striking elevations occurred in situations in which fetal death occurred before amniocentesis, elevated and rising AF Osm was also observed to precede subsequent fetal death or serious perinatal compromise. More recent data from this laboratory in a

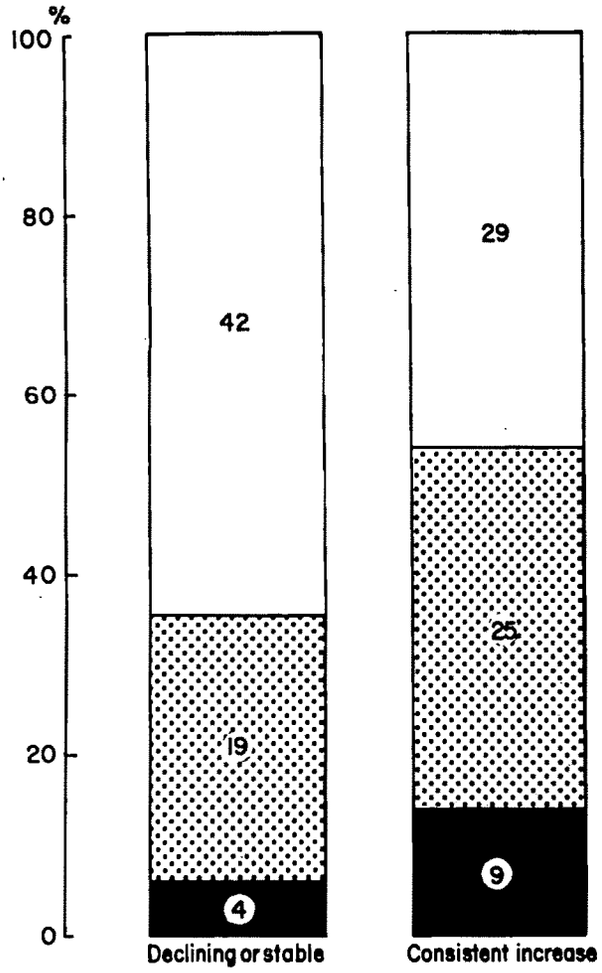


Fig. 2. Relation of serial amniotic fluid osmolality to perinatal outcome. Number of pregnancies studied indicated in each block. Solid block, perinatal death; stippled block, perinatal morbidity (see text for definition); clear block, no morbidity or deaths.

total 156 Rh-sensitized pregnancies have confirmed these earlier serial studies.[7] The work of O'Leary and Feldman[5] is difficult to interpret because of their neglect to analyze their data statistically. AF Osm evaluations from a total 16 diabetic and 81 Rh-sensitized pregnancies (nine with fetal death) were compared to those from 87 normal pregnancies. Although mean AF Osm at 32 to 35 and 36 to 37 weeks' gestation was consistently higher (by as much as 9 mOsm. per kilogram) in the complicated pregnancies, the authors dismissed the clinical value of these findings and their data as reported do not allow a more careful statistical analysis. Kittrich and associates[6] showed a threefold increase in perinatal asphyxia in gestations with AF Osm >275 mOsm. per kilogram.

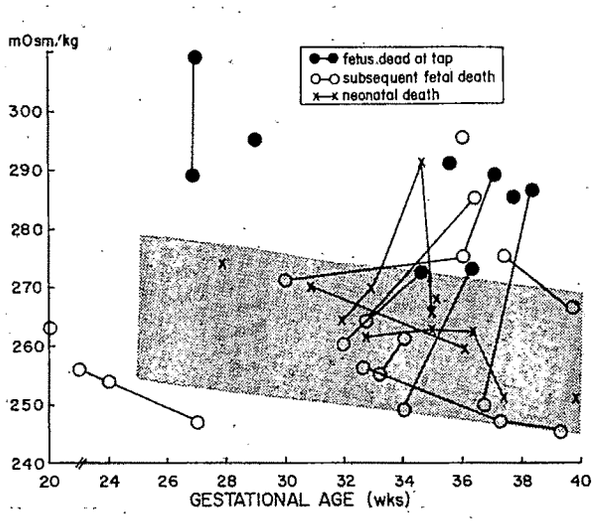


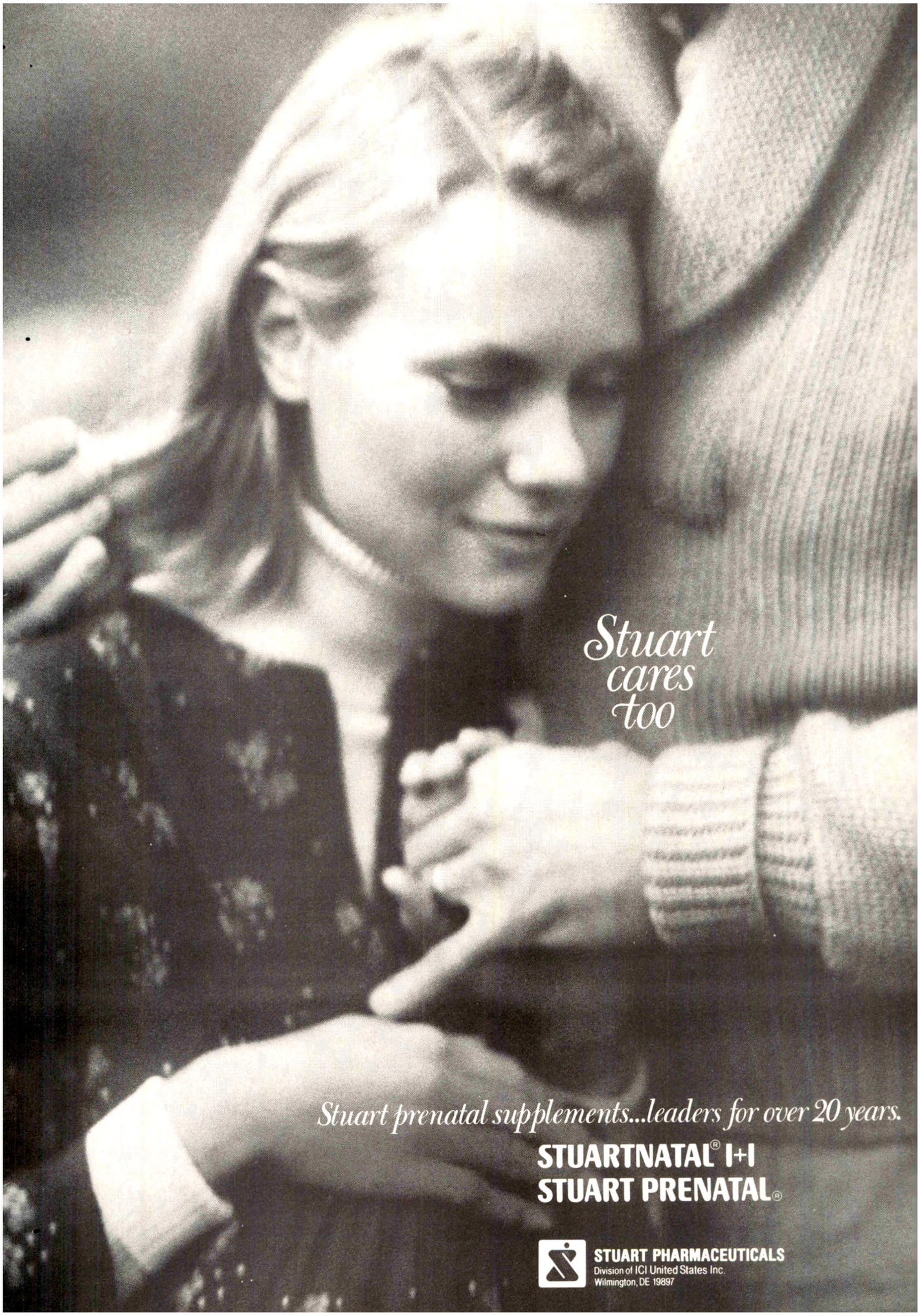
Fig. 3. Relation of amniotic fluid (AF) osmolality to gestational age in 13 pregnancies in diabetic women terminating in perinatal death. Individual AF osmolalities overplotted on normal range (±2 s·y·x) of AF osmolality (stippled area).[2]

Pregnancies complicated by diabetes and postmaturity were significantly more common in their group with "hypertonic" amniotic fluid. Seventeen of 22 pregnancies complicated by diabetes had hypertonic amniotic fluid at delivery.

There seems to be little doubt that increased AF Osm may accompany perinatal compromise. Fluids in these at-risk groups, however, are often obtained early in gestation.[7] AF Osm is inversely related to gestational age, with an expected decline of about 1 mOsm. each week.[2] Balancing the present data for gestational immaturity reveals that significant elevations of mean AF Osm accompany perinatal death. Severity of maternal diabetes, other than increasing the risk of perinatal death, does not directly affect AF osmolality, nor do maternal vascular disease or conditions resulting in altered fetal growth, perinatal asphyxia, or neonatal hypoglycemia. Of particular clinical pertinence are the observations that abnormal serial increases in AF Osm during the course of pregnancy complicated by diabetes accompany a significant increase in risk of perinatal compromise (14 per cent mortality rate and cumulative morbidity and mortality rate of 54 per cent). These data suggest that while serial evaluations of AF osmolality do not invariably predict the unsuccessful outcome of a pregnancy complicated by maternal diabetes (six per cent death and 35 per cent morbidity in patients with "normal" declines in AF Osm), the serial evaluation of amniotic fluid solute content does distinguish a "higher risk" group in which perinatal death is more than doubled.

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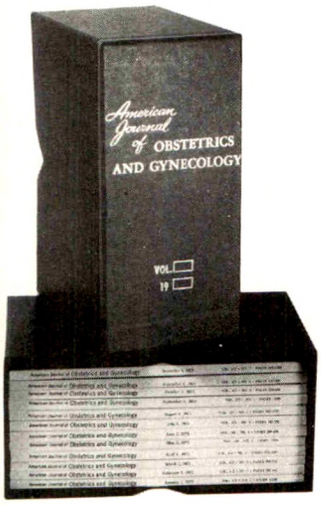
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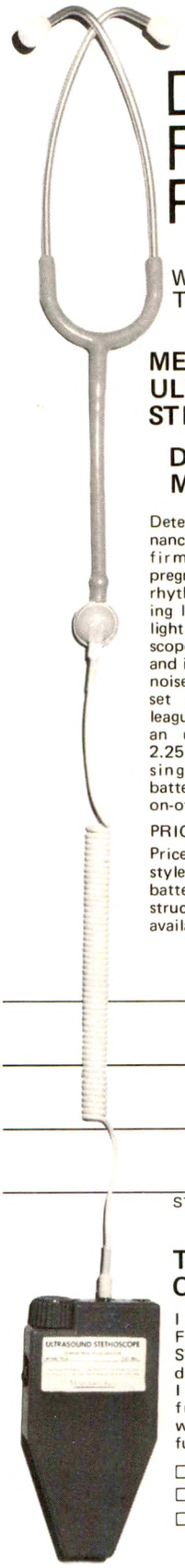
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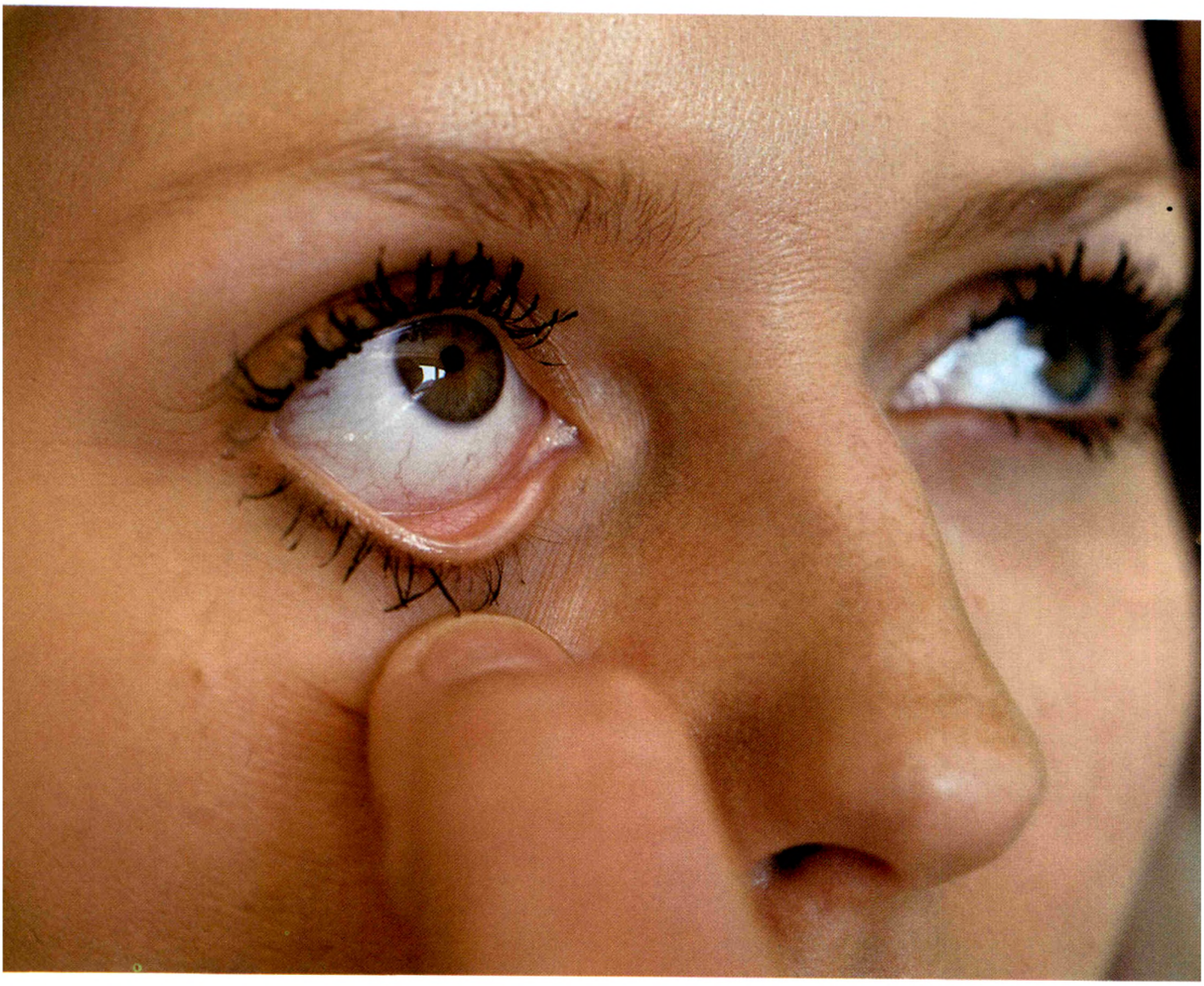
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CONTRAINDICATION: Pernicious anemia.

WARNINGS: Folic acid alone is improper therapy in the treatment of pernicious anemia and other megaloblastic anemias where vitamin B_{12} is deficient.

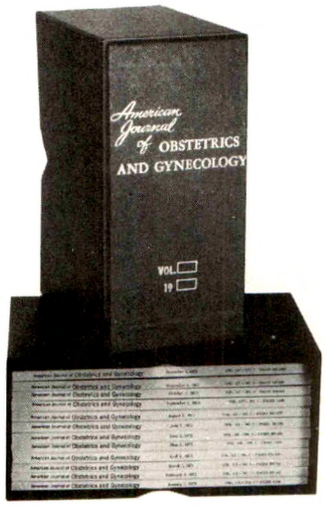
PRECAUTION: Where anemia exists, its nature should be established and underlying causes determined. Iberet-Folic-500 contains 800 mcg. of folic acid per tablet. Folic acid especially in doses above 1.0 mg. daily may obscure pernicious anemia, in that hematologic remission may occur while neurological manifestations remain progressive.

ADVERSE REACTIONS: The likelihood of gastric intolerance is remote. If such should occur, the tablet may be taken after a meal. Allergic sensitization has been reported following both oral and parenteral administration of folic acid.

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Articles to appear in early issues

Contraceptive knowledge and practice among undergraduates at a Canadian university
Diane Munz, B.A., Stephen Carson, B.Sc., Barry Brock, B.Eng., Lorraine Bell, B.Sc., Irwin Kleinman, B.Sc., Marie Robert, B.Sc., and Janine Simon, B.A.
Montreal, Quebec, Canada

Second-trimester spontaneous abortion, the IUD, and infection
Steven H. Eisinger, M.D.
Madison, Wisconsin

Effects of fasting and theophylline on alanine-stimulated glucagon secretion in neonatal and infant sheep
R. H. Fiser, Jr., M.D., D. A. Fisher, M.D., P. R. Williams, M.D., M. A. Sperling, M.D., and W. Oh, M.D.
Torrance, California

Incidence of cervical cancer in perimenopausal and postmenopausal women detected by Papanicolaou smears
Ralph M. Neighbor, M.D., and Robert L. Newman, M.D.
Kansas City, Missouri

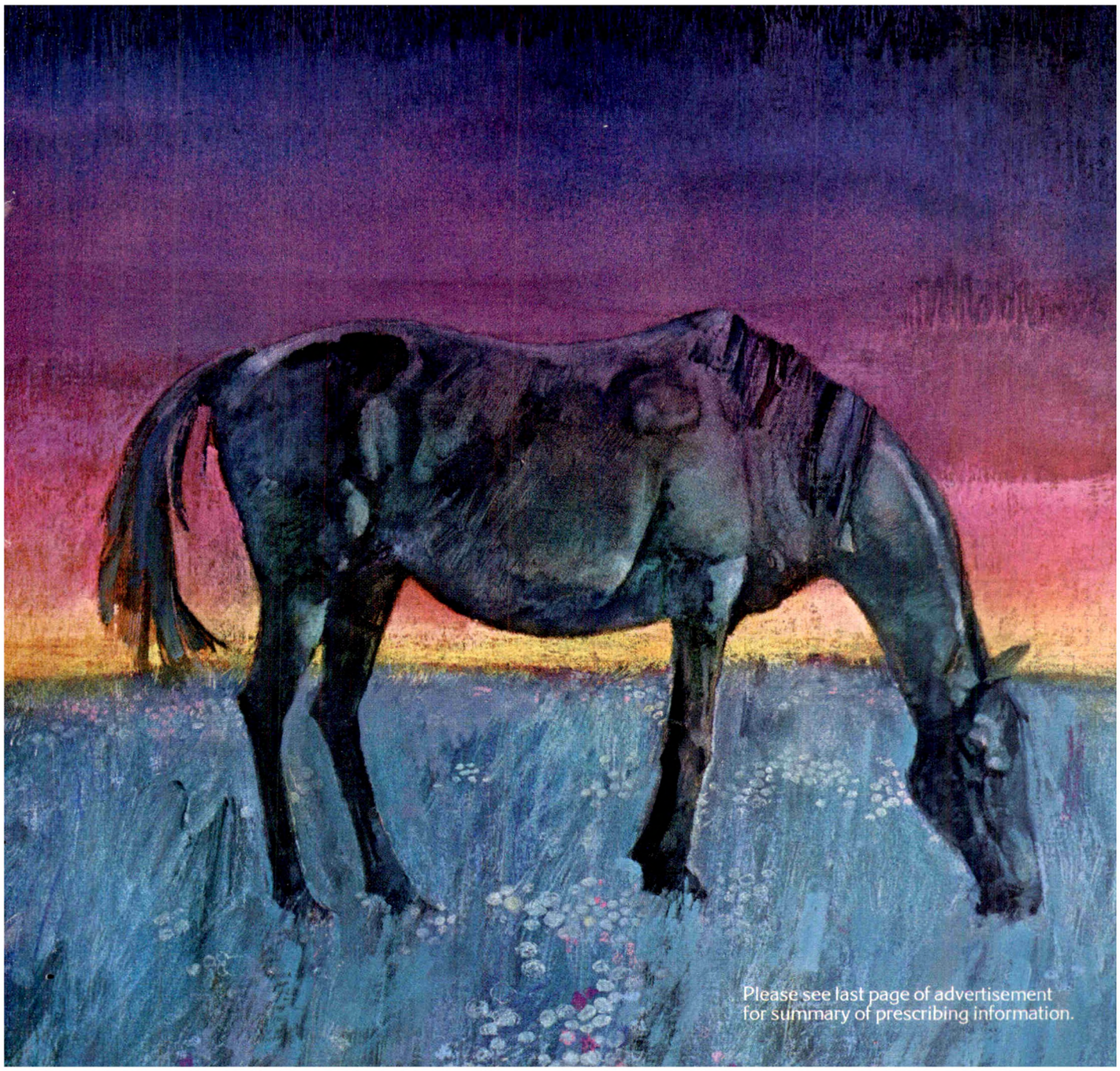
Epidermoid cysts of the ovary: A report of five cases with histogenetic considerations and ultrastructural findings
Francisco F. Nogales, Jr., M.D., and Steven G. Silverberg, M.D.
Denver, Colorado

Studies on the cytodynamics of human endometrial regeneration. II. Transmission electron microscopy and histochemistry
Alex Ferenczy, M.D.
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Histologic characteristics of cells cultured from rat placental tissue
F. G. Ferguson and J. Palm
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The influence of aspirin on the course of induced midtrimester abortion
Jennifer R. Niebyl, M.D., David A. Blake, Ph.D., Lonnie S. Burnett, M.D., and Theodore M. King, M.D., Ph.D.
Baltimore, Maryland

The obstetrician's role in reducing the risk of aspiration pneumonitis
Robert B. Roberts, M.D., F.F.A.R.C.S., and Michael A. Shirley, M.D.
New York, New York


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for summary of prescribing information.



The dawn of a new age

Genisis—The Reason for Change

Discovery, isolation, identification, synthesis—the classical pattern for creating drugs which once were available from natural sources only. Antibiotics, vitamins, and sex hormones come to mind as examples of drugs which have been developed and perfected in this manner. Obvious advantages of this evolution include: economy, standardization, reproducibility, and purity.

Natural Estrogens—A Mystique

There are those who would have us believe that conjugated estrogens, U.S.P. derived from the urine of pregnant mares is endowed with a certain mystique. What, precisely, is conjugated estrogens "natural"?

A study of the excretion pattern of pregnant-mare estrogens reveals that estrone and equilin are the major components and 17 alpha-dihydroequilin a minor one. Estrone predominates in early pregnancy but as this progresses equilin levels increase and may equal or surpass those of estrone in late pregnancy. This is believed to reflect continuing changes in the placental metabolism of estrogen.[1]

The urine of pregnant mares is pooled to compensate for these variations and provide a raw material of more or less average composition. This practice, however, has the disadvantage of providing a urine pool which contains all of the excreted estrogenic and related hormones whether active or not. For example, an assay study of the estrogenic content of a "large mixture" of mares' urine reveals the following proportions: 51% sodium estrone sulfate, 22% sodium equilin sulfate, 16% sodium 17 alpha-dihydroequilin, and smaller amounts of the sodium sulfates of equilenin, 17 alpha-dihydroequilenin and 17 alpha-estradiol. Related "non-estrogenic" steroids were also present.[2]

Despite these variations, conjugated estrogens, U.S.P. is defined as containing 50 to 65% sodium estrone sulfate and 20 to 35% sodium equilin sulfate.[3]

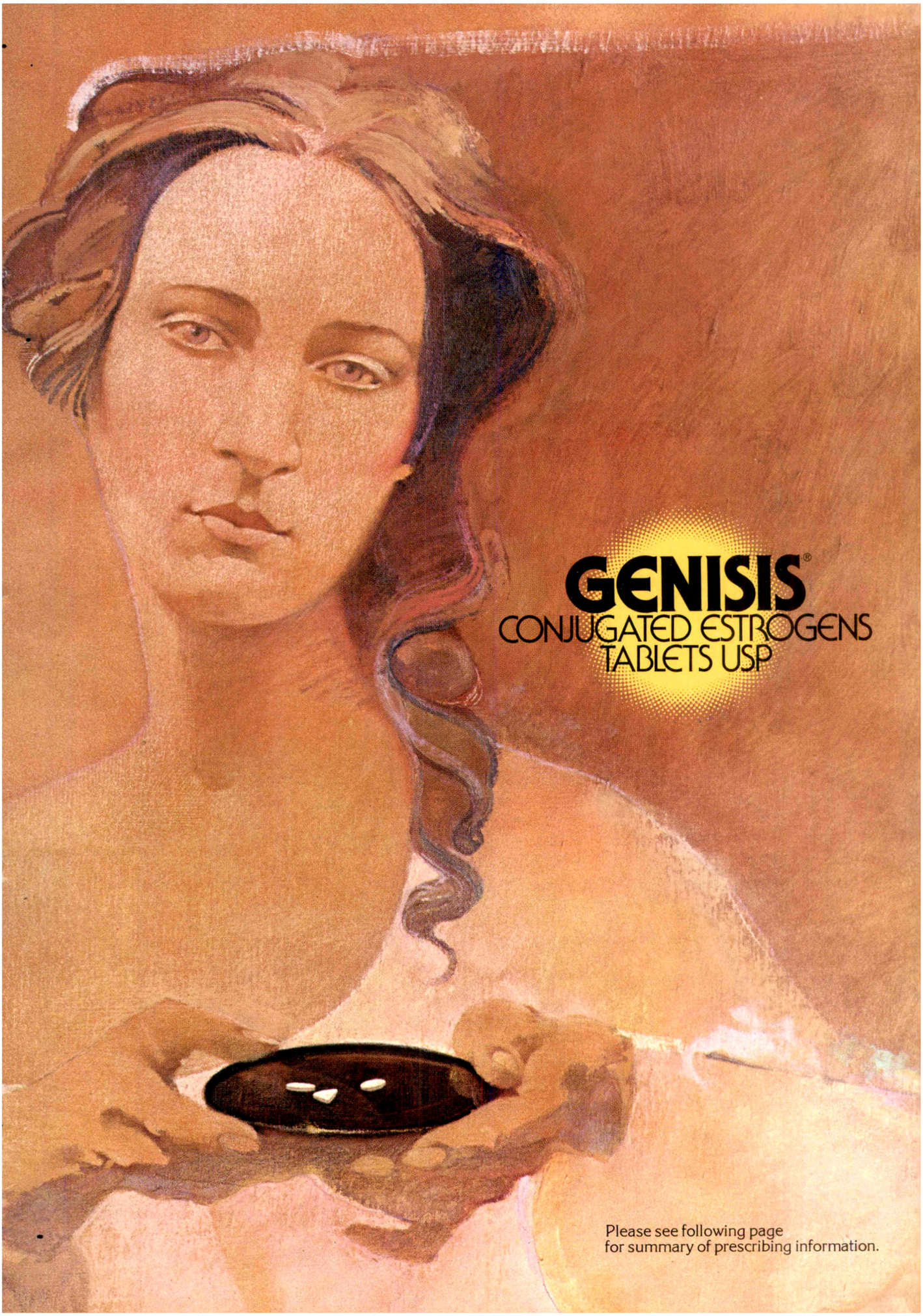
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Genisis is indicated for those standard indications requiring conjugated estrogens.

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BRIEF SUMMARY
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Indications
Genisis is indicated for replacement therapy of estrogen deficiency associated with menopausal syndrome, female hypogonadism (hypogenitalism), amenorrhea, female castration or primary ovarian failure.

Important Notes on the Use of Estrogens
In view of the positive relationship between thromboembolic disease and the use of oral contraceptives containing estrogens and progestins, it would seem prudent and in keeping with basic therapeutic principles to utilize, whenever feasible, the smallest effective dose of estrogens in treating patients.

The possible carcinogenicity of estrogens cannot be confirmed or refuted at this time. Close clinical surveillance of all women taking estrogens must be continued.

Contraindications
Patients with, or with history, of:
markedly impaired liver function;
known or suspected carcinoma of breast (except progressing disease not amenable to surgery or irradiation in women at least 5 years postmenopausal, or in men in whom castration is not possible);
known or suspected estrogen-dependent neoplasia, such as carcinoma of the endometrium;
undiagnosed genital bleeding;
thrombophlebitis, thromboembolic disorders, cerebral apoplexy.

Warnings
Discontinue medication at the earliest sign of thrombotic disorders (thrombophlebitis, cerebrovascular disorders, pulmonary embolism, retinal thrombosis), or in the presence of papilledema, retinal vascular lesions or such untoward effects as progression of cancer or hypercalcemia. Discontinue pending examination if there is sudden onset of proptosis, diplopia or migraine.

A statistically significant association has been reported between maternal ingestion of diethylstilbestrol during pregnancy and the occurrence of vaginal carcinoma years later in the offspring. Whether such an association is applicable to all estrogens is not known at this time. In any event, estrogens are not indicated for use during pregnancy.

Estrogens excreted in mother's milk may affect the nursing infant.

Precautions
Certain patients may be unusually responsive and may respond with undesirable manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding, mastodynia, edema, etc. Because of possible salt and water retention, estrogens should be used with caution in patients with epilepsy, migraine, asthma, cardiac or renal disease. Estrogens influence the metabolism of calcium and phosphorus. They should be used with caution in patients with certain metabolic bone diseases that are associated with hypercalcemia or in patients with renal insufficiency. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Diabetic patients should be followed closely because of possible decrease in glucose tolerance.

In the event of unexplained or excessive vaginal bleeding during estrogen therapy, nonfunctional causes should be borne in mind. The drug should be discontinued and a thorough investigation made as to the cause, being certain to rule out the possibility of malignancy. Pre-existing uterine fibromyomata may increase in size when estrogens are used. Therefore, patients should be examined at regular intervals throughout therapy. Women with a strong family history of cancer, recurrent chronic mastitis, or abnormal mammograms should be administered estrogens with caution. A pre-treatment physical examination should include special reference to breast and pelvic organs as well as a Papanicolaou smear since estrogens have been known to produce tumors, some of them malignant, in five species of animals.

Continuous use of estrogens will result in prolonged stimulation of the endometrium and breast. In order to avoid this, oral estrogens should be administered cyclically in the menopausal or hypogonadal patient.

The pathologist should be advised of estrogen therapy when relevant specimens are submitted. Certain endocrine and liver function tests may be affected by treatment with estrogens. If such tests are abnormal in a patient taking these drugs it is recommended that they be repeated after the drug has been withdrawn for two months. Any possible influence of prolonged estrogen therapy on pituitary, ovarian, adrenal, hepatic or uterine function awaits further study.

When large doses of estrogens are used, urinary stress incontinence may occur in nonpregnant females.

Adverse Reactions
A statistically significant association has been demonstrated between use of estrogen-progestin oral contraceptives and the following serious reactions: thrombophlebitis, cerebral thrombosis, pulmonary embolism.

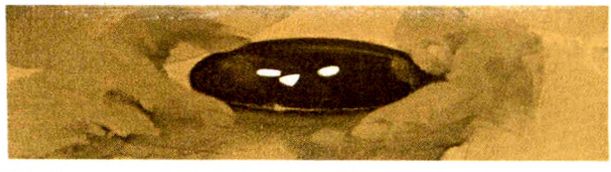
Although available evidence is suggestive of an association, such a relationship has been neither confirmed nor refuted for the following serious reactions: coronary thrombosis, neuro-ocular lesions (e.g. retinal thrombosis and optic neuritis).

The following adverse reactions are known to occur in patients receiving estrogens: nausea, vomiting, anorexia, gastrointestinal symptoms (such as abdominal cramps or bloating), edema, breakthrough bleeding, spotting or withdrawal bleeding, breast tenderness and enlargement, change in body weight (increase or decrease), headache, increased cervical mucus, allergic rash, loss of libido and gynecomastia in the male, reactivation of endometriosis, aggravation of migraine headaches, hepatic cutaneous porphyria becoming manifest, cholestatic jaundice, rise in blood pressure in susceptible individuals, mental depression, cystitis-like syndrome, loss of scalp hair, erythema nodosum, hemorrhagic eruption, premenstrual-like syndrome, changes in libido, changes in appetite, nervousness, dizziness, fatigue, backache, erythema multiforme, itching, possible diminution in lactation when given immediately post-partum, irritability, malaise.

Dosage and Administration
Dosage requirements will vary with the severity of menopausal symptoms. Initially, doses ranging from 1.25 mg to 3.75 mg daily may be required to bring symptoms under control. When this has been accomplished dosage should then be gradually decreased until the minimal effective maintenance dose is established. This will usually be 0.625 mg or 1.25 mg daily or possibly less.

Caution
Federal law prohibits dispensing without a prescription.

How Supplied—0.625 mg tablets—bottles of 100 and 500
1.25 mg tablets—bottles of 100 and 500
2.5 mg tablets—bottles of 100



References
1. Savard, K.: The estrogens of the pregnant mare. Endocrinology 68:411, 1961.
2. Howard, R.P. and Keaty, E.C.: Evaluation of equilin 3-monosulfate and other estrogens. Arch. Int. Med. 128:229, 1971.
3. Goodman, L.S. and Gilman, A.: The pharmacological basis of therapeutics. New York, MacMillan Pub. Co. Inc., 1975, p. 1431.

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FETUS, PLACENTA, AND NEWBORN

Rapid infusion of sodium bicarbonate and albumin into high-risk premature infants soon after birth: A controlled, prospective trial

RICHARD D. BLAND, M.D.
TERRELL L. CLARKE, M.D.
LEWIS B. HARDEN, M.D.
Honolulu, Hawaii

We conducted a controlled, prospective trial to evaluate the effectiveness of rapidly infusing sodium bicarbonate ($NaHCO_3$) and salt-poor albumin into high-risk, premature infants in the first 2 hours of life. Fifty-three infants, randomized into one of four treatment groups, received 8 ml. per kilogram of a solution containing either (A) glucose in water, (B) salt-poor albumin, (C) $NaHCO_3$, or (D) a combination of albumin and $NaHCO_3$. After the initial infusion, the babies received no colloid or alkali solutions until 4 hours of age. We managed them supportively with warmth, appropriate oxygen administration, isotonic fluid infusion, and close monitoring. Among the infants who received alkali, 14 of 26 acquired the respiratory distress syndrome (RDS), 11 died, and four had intracranial hemorrhage. Among babies who received no alkali, RDS occurred in 11 of 27, 5 died, and none had intracranial hemorrhage. These results do not support the common practice of rapidly infusing $NaHCO_3$ into high-risk, premature infants, and they suggest that the early management of such infants needs renewed critical evaluation.

OBSTETRICIANS, pediatricians, and anesthesiologists have infused hyperosmotic solutions of $NaHCO_3$ into newborn infants with acidemia for more than a decade. Reports by Hutchison and associates[1] and by Roberts and co-workers[2] supported the effectiveness of this practice in reducing the incidence of RDS and death, but neither included a suitable control group. In a controlled, prospective trial, Sinclair, Engel, and Silverman[3] were unable to demonstrate any benefit from rapid infusions of alkali to infants of low birth weight. The impact of that study, however, was limited by the small number of patients in each treatment group. A subsequent trial of early vs. delayed treatment of acidemia in low-birth-weight infants by Hobel and associates[4] demonstrated no difference between groups for incidence of RDS or related death. That study did not include a control group of infants from whom alkali was withheld.

Two retrospective studies carried out over the past decade have associated rapid infusions of $NaHCO_3$ with an increase in the mortality rate, accompanied by a high incidence of intracranial hemorrhage.[5, 6] Several editorial comments have ably outlined the controversy

From the Department of Pediatrics, Tripler Army Medical Center.

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which prevails over this therapeutic approach and have stated the need for further controlled, prospective trials.[7-10] This report describes the results of such a trial and is intended to provide some rationale for further evaluating therapy of acidemic premature infants.

Clinical population and methods

From October, 1970, to July, 1971, at Tripler Army Medical Center in Hawaii, we conducted a prospective study to assess the usefulness of infusing various solutions into the umbilical vein of high-risk infants shortly after birth. All infants admitted to the study were hypoproteinemic (cord serum total protein level of 4.6 Gm. per 100 ml. or less) and premature (less than 37 weeks' gestation). Previous experience in our nursery had shown us that RDS occurs in over 50 per cent of such infants, with a mortality rate of 34 per cent.[11]

The infants were weighed immediately on admission to the nursery, and gestational age was assessed on the basis of maternal menstrual history and obstetric milestones, adjusted according to various physical[12] and neurologic[13] criteria for maturity. We gave Apgar scores to each infant at 1 and 5 minutes of age. A small amount of serum was extracted from the cord blood sample after centrifugation for rapid determination of the serum total protein concentration. We used a modified refractometric technique requiring only one drop of undiluted serum transferred by pipette onto a hand refractometer.*[14] We measured the pH of arterial or "arterialized capillary" blood, and in many cases we measured the Pao_2 and $Paco_2$ on a blood gas analyzer† within 10 minutes of sampling.

For each infant admitted to the study, a nurse selected a therapy card, randomly mixed in sets of four (one for each therapy group) and matched according to birth weight (divided into 500 Gm. intervals). The card instructed her to draw up one of the following solutions in a sterile 30 ml. syringe:

A: 8 ml. per kilogram of glucose in water (5 Gm. per 100 ml.)

B: 8 ml. per kilogram of salt-poor albumin‡ (25 Gm. per 100 ml.)

C: 3 ml. per kilogram of $NaHCO_3$ (7.5 Gm. per 100 ml.) and 5 ml. per kilogram of water

D: 1.5 ml. per kilogram of $NaHCO_3$ (7.5 Gm. per 100 ml.), 4 ml. per kilogram of salt-poor albumin (25 Gm. per 100 ml.), and 2.5 ml. per kilogram of water

We explained the nature of the study to one or both parents of each infant, and we obtained permission to include the infant in the trial. We then inserted a polyethylene catheter* into the baby's umbilical vein through the ductus venosus to the inferior vena cava, where we measured the venous pressure and infused the trial solution over a 5 to 10 minute period. In some cases, we were unable to advance the catheter through the ductus venosus, and in those patients we infused the solution through the portal venous system. We removed the catheter promptly and if there was any evidence of sustained distress, we inserted a peripheral venous line or an umbilical arterial catheter. In all cases, we completed the initial infusion before the infant was 2 hours old, usually in the first 30 minutes. We obtained a chest x-ray for each infant, and we measured arterial blood gases and pH at frequent intervals when the infant had sustained distress or required supplemental oxygen.

When admitted to the study, all infants breathed spontaneously. After the initial infusion, the patients received no colloid or alkali solution over the first 4 hours of life. We managed them supportively with warmth, appropriate oxygen administration, isotonic fluid infusion, and close monitoring. The study took place prior to the use of continuous positive airway pressure or ventilation with positive end-expiratory pressure in our nursery.

We diagnosed RDS on the basis of a sustained respiratory rate greater than 60 per minute, repeated observations of xiphoid and rib retractions, an expiratory grunt, and cyanosis without oxygen, with symptoms for at least 72 hours or until death, thereby excluding cases of transient neonatal tachypnea. All infants with RDS had x-ray evidence of diminished aeration with "air bronchograms." Patients who died had postmortem examinations within 24 hours of death.

Results

Table I demonstrates the striking similarity in major characteristics of the four groups of infants. By the unpaired t test, there was no significant difference between groups for gestational age, birth weight, initial pH, venous pressure, Apgar scores, or serum protein concentration of the cord blood. While all infants who received $NaHCO_3$ alone were male, it is noteworthy

*TS meter, model No. 10400: American Optical Corporation, Scientific Instrument Division, Buffalo, New York.

†Blood gas analyzer, model No. 127: Instrumentation Laboratory, Inc., Boston, Massachusetts.

‡Albumin, Normal Human Serum, USP, Salt Poor: Metrix, Clinical and Diagnostics Division, Armour Pharmaceutical Company, Chicago, Illinois.

*Argyle umbilical artery catheter, 5 Fr., model No. MAR1602-5: Sherwood Medical Industries, Inc., St. Louis, Missouri.

Table I. Major characteristics of the four groups of infants*

	Nonalkali groups		*Alkali groups*	
	A	*B*	*C*	*D*
No. of infants	13	14	13	13
No. of males	10	7	13	4
Gestational age (wk.)	34.8 (0.5)	32.3 (1.0)	33.8 (0.7)	33.4 (0.7)
Birth weight (Gm.)	2,149 (122)	1,791 (161)	2,075 (124)	1,982 (136)
Initial pH	7.28 (0.03)	7.25 (0.03)	7.23 (0.03)	7.27 (0.03)
Apgar score 1 min.	6.4 (0.6)	6.0 (0.6)	6.3 (0.8)	6.3 (0.7)
Apgar score 5 min.	7.7 (0.4)	7.1 (0.5)	7.6 (0.8)	6.2 (0.7)
Initial venous pressure (cm. H_2O)	4.6 (0.7)	4.8 (0.7)	4.0 (0.9)	4.2 (0.9)
Cord blood serum total protein concentration (gm./100 ml.)	4.0 (0.1)	4.0 (0.1)	3.9 (0.1)	4.0 (0.1)

*Group A received 8 ml. per kilogram of glucose in water; Group B received 8 ml. per kilogram of salt-poor albumin; Group C received 3 ml. per kilogram of $NaHCO_3$ and 5 ml. per kilogram of water; Group D received 1.5 ml. per kilogram of $NaHCO_3$, 4 ml. per kilogram of albumin, and 2.5 ml. per kilogram of water. We list average values, with standard error of the mean (S.E.M.) in parentheses.

that the sex distribution was virtually identical for the infants who received no $NaHCO_3$ (Groups A and B) and for those who did (Groups C and D).

Acidemia and respiratory distress, while present in the majority of infants, were not essential prerequisites for admission to this trial. Yet no infant in the $NaHCO_3$-infusion group had a pH in excess of 7.34, and only two had pH values greater than 7.30 before receiving alkali.

We observed the outcome of our management, looking primarily at the incidence of RDS, survival, and intracranial hemorrhage. The results are shown in Table II. Combining the infants who received $NaHCO_3$ (Groups C and D), 14 of 26 acquired RDS, 11 died, and four had intracranial hemorrhage (two intraventricular and two extensive subarachnoid). Among those babies who did not receive $NaHCO_3$ (Groups A and B), RDS developed in 11 of 27, five died, and there were no cases of intracranial hemorrhage. The likelihood that this distribution of deaths could have occurred by chance alone is <0.12 with chi-square analysis and the 2 × 2 contingency table corrected for continuity.[15]

Among those infants who remained in distress and required repeated measurements of arterial blood gases within the first 12 hours of life, the $Paco_2$ was higher in the patients who received $NaHCO_3$ than in those managed without alkali infusions, as shown in Table III ($p < 0.01$, by unpaired t test). While the average postinfusion pH was lowest in the group that received $NaHCO_3$ alone, the difference was not significant.

Table II. Outcome of infants in the four groups

	Nonalkali groups		*Alkali groups*	
	A	*B*	*C*	*D*
No. with RDS	5	6	8	6
No. died	1	4	6	5
No. with intracranial hemorrhage	0	0	3	1

Table III. pH and $Paco_2$ measurements of infants who remained in distress and required repeated determinations of arterial blood gases within the first 12 hours of life

	Nonalkalki groups		*Alkali groups*	
	A	*B*	*C*	*D*
No. of infants	8	11	9	10
Postinfusion $Paco_2$ (torr) (Mean and S.E.M.)	41 (4)	32 (3)	46 (4)	49 (4)
Postinfusion pH (Mean and S.E.M.)	7.32 (0.03)	7.35 (0.02)	7.29 (0.04)	7.33 (0.02)

Table IV summarizes data for the four infants who had intracranial hemorrhage at postmortem examination. As a group, they were lighter (average 1,549 grams), less mature (average gestation 31.8 weeks), and in more distress soon after birth (average Apgar scores 3.7 at 1 minute and 6.3 at 5 minutes, average initial pH 7.19) than the study patients taken together. The $Paco_2$ of all four infants was >50 torr after the initial infusion, and the pH rose appreciably in but two of the four babies after they received $NaHCO_3$-containing solutions. Another infant in the $NaHCO_3$ group died at 36 hours of age with massive pulmonary hemorrhage but had no evidence of bleeding into the brain.

Comment

Usher[16] began administering $NaHCO_3$ in dilute solutions and by slow intravenous infusion to newborn infants in 1958. Five years later, Dawes and his colleagues found that asphyxiated newborn lambs and rhesus monkeys resuscitated with hyperosmotic intravenous solutions of alkali and glucose recovered more quickly and had less residual brain damage than animals given no intravenous fluids.[17–19] There were no control animals who received isotonic fluids, and the authors were careful to warn against the application of

Table IV. Data for the four infants in whom we found intracranial hemorrhage at postmortem examination

Infant	*Sex*	*Group*	*Weight (Gm.)*	*Gestation (wk.)*	*Apgar score*		*Cord serum protein (Gm./100 ml.)*	*Venous pressure (cm. H_2O)*	*pH_1**	*pH_2†*	*$Paco_2$‡ (torr)*	*Site of hemorrhage*	*Time of death (hr.)*
					1 min.	*5 min.*							
1	M	C	1,505	32	?	7	3.2	0	7.27	7.28	50	Subarachnoid	40
2	M	C	1,600	29	1	2	4.2	3	7.05	6.90	55	Subarachnoid	3
3	M	C	1,270	32	6	8	3.2	4	7.18	7.24	50	Ventricles	20
4	F	D	1,820	34	4	8	4.4	2	7.25	7.33	60	Ventricles	10

*pH_1 is the pH measured in the first 2 hours of life, before the trial solution was given.
†pH_2 is the pH measured after the solution was given, in all cases within 4 hours of the infusion.
‡$Paco_2$ was measured after the infusion.

this approach to the management of human newborn infants without supportive clinical data.

Hutchison and associates[1] reported that rapid infusions of alkali improved the survival rate of infants afflicted with RDS and recommended this mode of therapy for all premature infants with acidemia. That same report described but neglected to discuss a substantial increase in the incidence of intracranial hemorrhage among infants treated with rapid infusions compared with a previous group treated more conservatively. More recent observations by Usher[5] and by Simmons and associates[6] have confirmed this association.

Finberg[20] attributed this relationship to abrupt changes in serum osmolality induced by such infusions, with rapid shifts in fluid from the intracellular to the extracellular compartment. He based his explanation on the finding of hemorrhagic encephalopathy in cats given large volumes of hyperosmotic solutions.[21, 22] If this mechanism is responsible for producing hemorrhage, infusion of salt-poor albumin should have a comparable effect by inducing similar fluid shifts from the intracellular space. Yet we did not find an association between infusions of albumin and intracranial hemorrhage.

Several investigators have shown that administration of hypertonic solutions may produce a fall in pH resulting from dilutional effects on extracellular buffers and release of protons from intracellular buffers.[23–25] Though we diluted the $NaHCO_3$ which we infused to an osmolality of approximately 550 mOsm. per liter, we were unable to show a sustained elevation of arterial blood pH among infants given $NaHCO_3$. This finding was, at least in part, the result of CO_2 retention, and emphasizes the importance of adequate ventilation in correcting the acidemia of RDS. Holmdahl and associates,[26] and more recently Steichen and Kleinman,[27] showed that in the presence of fixed ventilation (as we might see in premature infants with the most severe RDS), infusions of $NaHCO_3$ may produce a rise in $Paco_2$. Since hypercarbia induces cerebral vasodilatation,[28] is it possible that this effect of $NaHCO_3$ infusions, without respiratory adjustment, particularly in the presence of elevated venous pressure, might induce intracranial hemorrhage?

The usual rationale for infusing alkali into newborn infants with acidemia is that pulmonary vascular resistance varies directly with hydrogen ion concentration,[29] and a sudden rise in arterial pH improves pulmonary blood flow and increases Pao_2.[30, 31] Johnson and co-workers[32] found, however, that the effects of hyperosmotic $NaHCO_3$ on the pulmonary vascular resistance of fetal lambs may not be related strictly to pH, but may reflect changes in circulating volume.

Rapid infusions of $NaHCO_3$ produce a small, transitory rise in arterial blood pressure, serum sodium concentration, and osmolality, with a fall in packed red blood cell volume.[33] These changes are consistent with a transient increase in circulating blood volume, similar to the changes which occur in cats after rapid infusion of hypertonic saline.[22] It might be argued then that any physiologic benefit derived from infusion of $NaHCO_3$ is related simply to intravascular volume expansion.

It was this rationale which led us to compare infusions of solutions which merely maintain intravascular volume to those which alter blood pH and osmolality. We initiated the trial as a pilot study, and perhaps it is unfortunate that we turned our attention to evaluating the effectiveness of albumin infusions vs. glucose in water[34] before reaching a more definitive answer to the issue regarding $NaHCO_3$ and intracranial hemorrhage.

The groups in this trial, though randomized, were small, and the data were limited, with no consistent measurements of serum sodium concentration or arterial blood pressure. Some infants who received $NaHCO_3$ were not severely acidemic, and some who received albumin probably were not hypotensive, though all had low serum protein concentrations. These shortcomings aside, the incidence of RDS and

death was higher in the alkali-treated groups than in the nonalkali groups, and there were four cases of intracranial hemorrhage found in those infants who received $NaHCO_3$ vs. no cases among babies who received glucose in water or albumin. These results do not support, in any way, the usefulness of rapid infusions of $NaHCO_3$ to high-risk infants, and they suggest that early management of such infants needs renewed critical evaluation.

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Hyperfibrinogenemia and polycythemia with intrauterine growth retardation in fetal lambs

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Plasma concentrations of albumin and fibrinogen and arterial hematocrits were determined during the last third of gestation in growth-retarded and control fetal lambs. The mean fetal plasma albumin concentration increased slightly as term approached and was not significantly different in the two groups. The mean plasma fibrinogen concentration did not change in the control fetuses, but was significantly elevated in the growth-retarded fetuses, as was the mean arterial hematocrit. The theoretical implications of these findings relative to capillary blood flow are discussed.

ALTHOUGH intrauterine growth retardation is a relatively common clinical problem, very little is known about the influences that growth retardation may have on the biochemical and physiologic functions of the fetus or neonate.

We have produced fetal growth retardation in the fetal lamb by a gradual embolization of the uteroplacental vascular bed.[1] Changes in the plasma concentrations of fibrinogen and albumin and in the arterial hematocrit were measured. Hyperfibrinogenemia and polycythemia characterized the terminal portion of the gestation in these growth-retarded fetuses. The theoretical implications of these findings in relation to peripheral capillary blood flow are discussed.

Methods

Embolization of the maternal placental implantation site in pregnant sheep was performed as previously described.[1] Dated pregnant sheep were used in all experiments. In brief, catheters were inserted in fetal and maternal vessels at approximately 110 days of gestation. The catheters were exteriorized, and the pregnancies allowed to proceed normally. Alfalfa and water were provided *ad libidum* for the remainder of the experiment. Growth retardation in the developing fetus was produced by daily injection of nonradioactive microspheres (15μ diameter) into a catheter placed in the main uterine artery supplying the pregnant horn. Control animals were prepared in a similar manner but did not receive the embolization with microspheres. With this procedure, approximately 30 to 40 per cent of the total number of cotyledons are grossly abnormal, being reduced in thickness. Microscopic examination reveals extensive fibrosis and hyalinization.

Samples of fetal femoral arterial blood were obtained for biochemical and hematocrit determinations at intervals during the subsequent course of the gestation. Blood samples used for the biochemical assays were placed in citrated tubes and centrifuged, and the plasma was immediately pipetted and frozen at −20° C. The fetal plasma fibrinogen concentration was determined by the Saifer-Newhouse[2] method, the fetal plasma albumin was assayed by paper electrophoresis,[3] and the fetal arterial hematocrit was determined with a microcentrifuge.

The studies in both groups of animals were terminated at approximately 140 days of gestation and the fetal weight and organ weights determined for confirmation of intrauterine growth retardation.

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Table I. Fetal plasma fibrinogen concentration* in control and growth-retarded fetal lambs

Gestational age (days)	*Control fetuses*	*Growth-retarded fetuses*	*P values*
114-122	131 ± 24 (8)†	160 ± 71 (6)	0.05
123-129	143 ± 17 (5)	247 ± 89 (3)	0.025
130-140	118 ± 30 (11)	236 ± 127 (10)	0.0005

*Values are mean concentration in milligrams per 100 ml ± 1 S.D.

†Numbers in parentheses indicate number of fetuses.

Table II. Arterial hematocrit (means ± 1 S.D.) in control and growth-retarded fetal lambs at the onset and termination of the chronic study

	Controls	*Growth-retarded*
Onset	0.34 ± 0.02	0.35 ± 0.04
Termination	0.35 ± 0.04	0.44 ± 0.03*

*Significant difference at $p < 0.05$.

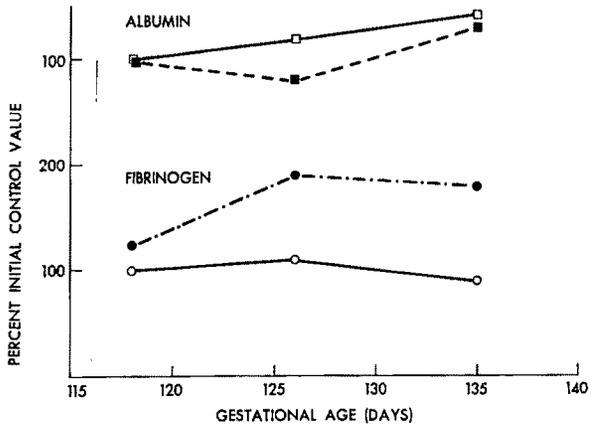


Fig. 1. Changes in plasma albumin (squares) and fibrinogen (circles) concentrations during gestation in fetal sheep. Growth-retarded fetuses are denoted by interrupted lines. Differences between normal and growth-retarded fetuses at the ages tested were not significant for albumin and highly significant for fibrinogen (see Table I.).

The unpaired t test (two-tailed) was used to check for significant differences between the control and embolization groups.

Results

The results of the embolization procedure in regard to detailed anatomic and physiologic data have been previously reported.[1, 4] In brief, this procedure results in fetuses which are significantly smaller in weight and length. Most fetal organs are reduced in size, particularly the liver and thymus. Fetal Po_2 is reduced 15 to 25%.[1, 4] The fetuses in the present embolization group showed similar evidence of fetal growth retardation.

The mean fetal plasma fibrinogen concentration in the control group remained relatively constant between 114 and 140 days of gestation, as shown in Table I. The mean fetal plasma fibrinogen in the growth retarded fetuses was significantly increased over the control group.

Small but statistically significant differences in plasma fibrinogen concentrations were noted in the earlier sampling period (114 to 122 days). The differences in fibrinogen concentration between control and growth-retarded fetuses became more pronounced as gestation progressed, as shown in Fig. 1.

The plasma albumin concentrations in the control group increased in a linear manner as gestation advanced and were not significantly different from those of the growth-retarded fetuses.

At the onset of the study the mean fetal hematocrits were similar, as shown in Table II. At the end of the study the mean fetal hematocrit in the control group was 0.35 ± 0.04, and 0.44 ± 0.03 in the growth-retarded fetuses—a significant difference.

Comment

The results indicate that embolization of the maternal uteroplacental vascular bed is associated with an increase in the concentration of plasma fibrinogen and the arterial hematocrit of the fetal lamb. The increase in fibrinogen is not derived from maternal sources, as it is not transferred across the mammalian placenta,[5] and it is most unlikely that the increase in hematocrit is due to transfer of maternal red cells.

The polycythemia in the growth-retarded fetuses may be explained as a response to arterial hypoxemia found in these fetuses. The hyperfibrinogenemia may be explained as a result of fetal stress, as the concentration of the plasma clotting protein fibrinogen is an excellent indicator of stress in adult mammals. Various types of traumatic or toxic injuries, such as burns, crushing of extremities, and treatment with endotoxin, talcum powder, and turpentine, rapidly stimulate production of fibrinogen in the liver and increase the plasma fibrinogen concentration.[6] Even psychological stresses, such as electroshock[7] or crowding,[8] will produce a rise in circulating fibrinogen within 24 hours.

A number of studies on hyperfibrinogenemic states have indicated that the increased concentration of the protein is due to an accelerated hepatic synthesis rather than a decreased catabolism of fibrinogen.[6, 9] In these experimental growth-retarded fetuses an etiologic factor could have been the embolization process itself,

as intravascular clotting and formation of thrombin are known to increase the rate of hepatic fibrinogen synthesis.[10] Small peptides produced by the clotting mechanism have also been proposed as being able to stimulate synthesis of fibrinogen.[11] Although fibrinogen does not cross the placental barrier, it is conceivable that the small peptides could cross the placenta and affect the fetal liver. It is also interesting that the increase in fetal fibrinogen concentrations occurred despite an approximately one-third reduction in fetal liver weight.

In contrast with fibrinogen, the albumin concentrations in stressed fetuses in our experiments were either unaffected or depressed, but the decreases in albumin were not statistically significant. Similarly, Neuhaus and associates[12] have shown that in traumatized adult animals hepatic production of fibrinogen, seromucoid, and acute-phase globulin are increased, whereas albumin synthesis either is reduced slightly or remains unaltered. Thus the response of fetal sheep to placental embolization appears to be similar to that seen in stressed adult animals.

Sarcione[13] demonstrated that plasma proteins which are elevated during stress (e.g., acute phase protein, fetal alpha protein, fibrinogen) are characteristic of "fetal synthetic patterns." Sarcione suggested that de-repression of "fetal genes" in adult liver may be responsible for this effect. The possibility that the increased fibrinogen concentration seen in the growth-retarded lamb fetuses could conceivably reflect a retarded progression to adult plasma protein patterns deserves further investigation.

The in vivo flow of blood through the microcirculation has been shown to be of an intermittent and pulsative nature.[14, 15] At the velocities of blood flow, or shear rates, found in the capillaries, blood exhibits non-Newtonian flow characteristics, acting as a solid during a no-flow period and as a liquid during flow periods. These properties of capillary flow are due to the intermittent formation and dissolution of red blood cell–fibrinogen aggregates at low blood velocities (0.1 to 0.5 cm. per second) found in the microcirculation.[16] The propensity of human blood to form these aggregates appears to be a synergistic function of the plasma fibrinogen concentration and the hematocrit.

While there is only a weak interaction of fibrinogen and red blood cells in sheep and goats, most mammals studied demonstrate a strong interaction of the two factors, which markedly increases erythrocyte aggregation in the capillaries. In a human fetus, an increase in hematocrit and fibrinogen similar to that in the growth-retarded sheep fetus would theoretically be expected to greatly increase the clumping and sludging of blood in the capillary beds, with the potential of reducing oxygen tension and tissue perfusion.[16–19]

In an attempt to quantify the above interactions, Merrill[16] and co-workers have defined the force required to cause static blood to flow as the *yield shear stress* of blood. Yield shear stress alternately may be considered to represent the force necessary to disrupt the fibrinogen-erythrocyte aggregates formed in nonflowing blood. When the theoretical yield shear stress is calculated by the equations obtained by Merrill with the data from these studies, we find that a tenfold increase in yield shear stress would be expected to exist in blood of human fetuses if a similar situation in hematocrit and fibrinogen existed (see Appendix).

This synergistic interaction between polycythemic and hyperfibrinogenemic states could, in human subjects, exacerbate a number of factors which contribute to fetal distress. Clinically, sludging of blood has been associated with anoxic lesions of tissues, low blood flow rates, reduced cardiac output, increased vascular resistance, and congestive heart failure.[17, 20] These studies on fetal sheep suggest that an elevated blood fibrinogen may play a role in the frequent occurrence of life-threatening situations in growth-retarded human fetuses and newborn infants.

Appendix

Merrill[16] and co-workers measured the yield shear stress (Ty) of blood at the shear strain rates found in the microcirculation. The yield shear stress of human blood when the hematocrit ranges between 30 and 50 per cent is closely approximated by the equation:

$$Ty = A(H - 0.06)^3 \quad (1)$$

where the hematocrit is H, and A is a strong function of the fibrinogen concentration. The effect of plasma fibrinogen concentrations of 100 to 400 mg. per 100 ml. on Ty correlates with the equation:

$$Ty = BCf^2 \quad (2)$$

where Cf is the plasma fibrinogen concentration in mg. per 100 ml., and B is a constant which is a weak function of other plasma proteins.

These equations were used to estimate the increase in yield shear stress in the blood of growth-retarded fetuses over control fetuses in our experiments. If $A = BCf^2$, then substitution in Eq. (1) yields:

$$Ty = BCf^2(H - 0.06)^3. \quad (3)$$

In the last sampling interval (130 to 140 days) the mean hematocrit and fibrinogen levels were 33.7 per cent and 118 mg. per 100 ml. for the control fetuses, and 43.7 per cent and 236 mg. per 100 ml. for the

growth-retarded fetuses, respectively. From Eq. (3), these values give the ratio:

$$\frac{\text{Ty (retarded)}}{\text{Ty (control)}} = \left(\frac{236}{118}\right)^2 \left(\frac{0.437 - 0.06}{0.337 - 0.06}\right)^3 = 10.06.$$

Thus, the increase in yield shear stress of blood in growth-retarded fetuses compared with controls would be approximately tenfold.

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Estriol in pregnancy

IV. Normal concentrations, diurnal and/or episodic variations, and day-to-day changes of unconjugated and total estriol in late pregnancy plasma

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Normal values of unconjugated, total, and immunoreactive (measured without extraction) plasma estriol (E_3) have been determined from radioimmunoassay data obtained in 217 uncomplicated third-trimester pregnancies. Small but significant diurnal variations in unconjugated and total plasma E_3 have been observed in a study comprising 12 women who were hospitalized during late pregnancy for diabetes, toxemia, or placenta previa. The late morning decreases in unconjugated and the afternoon/evening decreases in total plasma E_3 concentrations, averaging some 10 to 15 per cent, were overshadowed by considerable episodic fluctuations and may thus be clinically irrelevant. Day-to-day changes of unconjugated and total plasma E_3 concentrations in late pregnancy were similar and smaller than changes in urinary E_3 measurements. The urinary E_3/creatinine ratio, however, reducing inadequacies of 24 hour urine collections, varied less than unconjugated or total plasma E_3. The data suggest that a decrease in unconjugated or total plasma E_3 must exceed 40 to 45 per cent of the mean of the three preceding determinations if it is to be considered a signal of fetal distress.

ADVANCES in radioimmunoassay technique facilitate routine determinations of unconjugated or total plasma estriol (E_3) during the third trimester of pregnancy in many clinical laboratories.[1] Whether or not plasma E_3 determinations are useful in assessing fetal status in high-risk pregnancies, however, remains to be established. Moreover, even the presence and magnitude of diurnal and/or episodic variations and day-to-day changes in plasma E_3 concentrations are a matter of dispute.[2–6] Knowledge of these parameters, however, appears to be a prerequisite for a meaningful clinical evaluation of plasma E_3 determinations as a diagnostic adjunct in complicated pregnancies. This study therefore was undertaken to establish normal values and confidence limits for plasma concentrations of unconjugated, total, and immunoreactive (measured in unextracted plasma) E_3 in uncomplicated third-trimester pregnancies and to delineate their diurnal and/or episodic variations as well as day-to-day changes in comparison with urinary E_3 excretion in patients with complicated pregnancies.

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Materials and methods

In order to determine normal third-trimester plasma E_3 concentrations, a single venous blood sample was drawn from 300 apparently healthy women between

29 and 40 weeks of gestation who were followed in our prenatal clinic. A total of 217 of these gravidas were delivered at the Los Angeles County–University of Southern California Medical Center, Women's Hospital, within 42 weeks of their last menstrual period, completed singleton pregnancies without complications, and had normal healthy babies with birth weights above the tenth percentile and 5 minute Apgar scores of 7 and above. Only the data obtained in these 217 uncomplicated pregnancies were utilized to establish the normal late-pregnancy plasma E_3 values reported in this paper.

Nine diabetic gravidas and three women with toxemia, all hospitalized and in late pregnancy, were included in a study to assess possible diurnal and/or episodic fluctuations of plasma E_3. Venous blood was drawn from these volunteers at 6 A.M., 9 A.M., 12 A.M., 4 P.M., 7P.M., 12 P.M., and again at 6 A.M. and urine was collected during each of the 6 time intervals.

Day-to-day changes in plasma E_3 concentrations were determined and compared with those observed in 24 hour urinary estriol excretion in 14 hospitalized patients whose pregnancy was complicated by diabetes mellitus (n = 11), placenta previa (n = 2), and toxemia (n = 1). Observation periods ranged from 6 to 31 days, averaging 13 days per patient. All patients delivered normal healthy babies without signs of distress during labor and delivery. All patients except the two with placenta previa had negative oxytocin challenge tests.[7] Thus, no decrease in plasma or urinary E_3 in response to significant fetal distress was expected to have occurred during the observation period. Twenty-four hour urine collections were begun and completed at 6 A.M.

Venous blood samples were collected into EDTA tubes. The plasma was separated immediately by centrifugation and stored at −15° C. until assayed. Unconjugated, total, and immunoreactive plasma E_3 were determined by radioimmunoassay (RIA) as previously described.[1, 8] In brief, unconjugated plasma E_3 was measured as follows: 500 c.p.m. of trititated E_3 was added as recovery standard to 0.5 ml. of plasma prior to extraction with 3 ml. of 12 per cent ethyl acetate in n-hexane, followed by a second extraction with 3 ml. of 40 per cent ethyl acetate in n-hexane. The latter extract was evaporated and redissolved in 0.4 ml. of 70 per cent ethanol, of which 0.20 ml. was subjected to liquid scintillation counting for recovery control and 20 μl, in triplicate, was submitted to RIA with the use of an antiserum against estriol-6-(O-carboxymethyl) oxime coupled to bovine serum albumin (E_3-6-CMO-BSA). Dextran-coated charcoal was used to separate bound and unbound tritiated E_3. The sensitivity of the assay

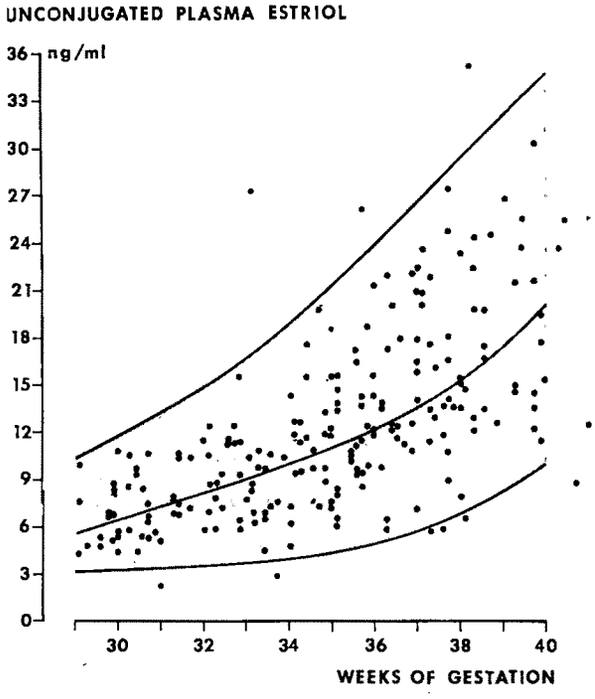


Fig. 1. Third-trimester unconjugated plasma estriol concentrations and 95 per cent confidence limits, calculated by Rankit analysis, in 217 women with uncomplicated pregnancies delivering normal healthy babies with birth weights above the tenth percentile. Note that the spread of values above the mean is greater than that below the mean.

was less than 1 ng. per milliliter, water and solvent blanks were undetectable, and intra- and interassay coefficients of variation (CV) were 10.7 and 9.5 per cent, respectively.

Total plasma E_3 was measured similarly by RIA.[1] In brief, 600 c.p.m. of tritiated estriol-3-sulfate-16-glucosiduronate (E_3-3S,16G)[9, 10] were added to 100 μl plasma aliquots as recovery standard. Following hot acid hydrolysis, the hydrolyzed E_3 was extracted with diethyl ether. Aliquots of the residue of the ether extract were subjected to RIA and to liquid scintillation counting to assess procedural loss. Water and solvent blanks were undetectable. A 5 ng per milliliter quantity was measured with a coefficient of variation of 10 per cent. The interassay CV was 6.8 per cent.

Immunoreactive E_3 was determined by subjecting 10 μl plasma aliquots directly to RIA with an antiserum against estriol-4-azobenzoic acid coupled to bovine serum albumin (E_3-4-ABA-BSA).[8, 11] This RIA was shown to determine unconjugated E_3 as well as estriol-3-sulfate (E_3-3S) and estriol-3-glucosiduronate (E_3-3G), but neither estriol-16-glucosiduronate (E_3-16G) nor E_3-3S, 16G.[1, 8] As little as 3 ng. per milliliter could be determined with a CV of 10 per cent. The interassay CV was 7.7 per cent.

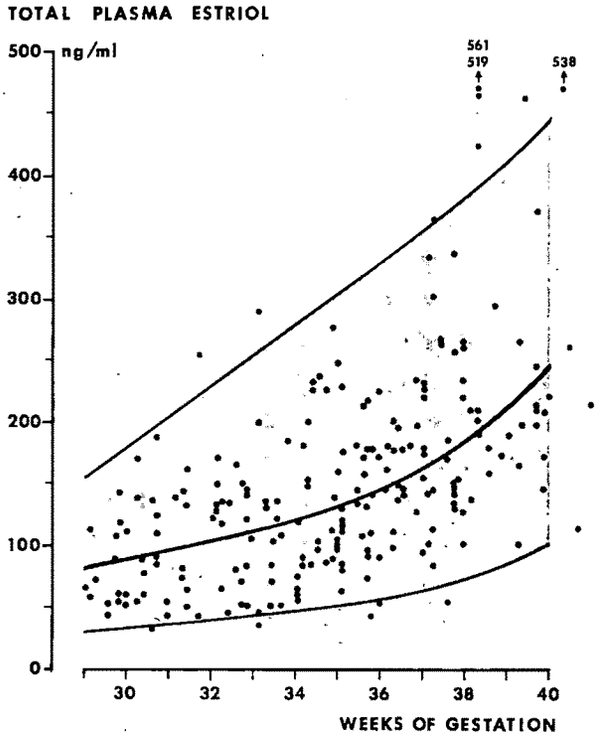


Fig. 2. Third-trimester total plasma estriol concentrations and 95 per cent confidence limits, calculated by Rankit analysis, in 217 women with uncomplicated pregnancies delivering normal healthy babies with birth weights above the tenth percentile. Note that the spread of values above the mean is greater than that below the mean.

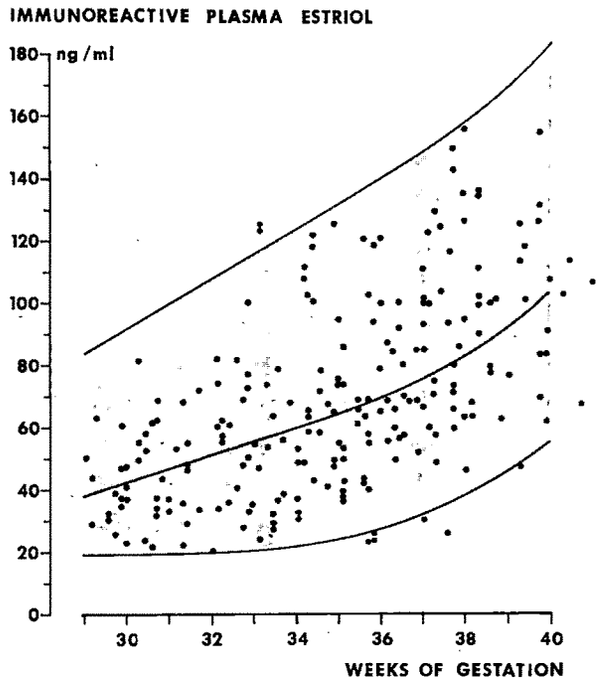


Fig. 3. Third-trimester immunoreactive plasma estriol concentrations and 95 per cent confidence limits, calculated by Rankit analysis, in 217 women with uncomplicated pregnancies delivering normal healthy babies with birth weights above the tenth percentile. Note that the spread of values above the mean is greater than that below the mean.

Urinary E_3 and creatinine were determined colorimetrically as previously described.[12]

Day-to-day changes in plasma total and unconjugated E_3 as well as in 24-hour urinary estriol excretion, as such and corrected for creatinine excretion, were analyzed by computer as previously described for urinary E_3.[12] Changes were expressed as increase or decrease in per cent of the mean of the three preceding determinations.

Results

Plasma unconjugated, total and immunoreactive E_3 concentrations in normal pregnancies. The concentrations of unconjugated, total, and immunoreactive plasma E_3 determined in 217 normal pregnancies are depicted in Figs. 1 to 3. Individual values were averaged for 2 week periods from 29 and 30 weeks up to 39 and 40 weeks. Statistical analysis revealed a larger spread of values above than below the means calculated for each 2 week period. The 95 per cent confidence limits shown in Figs. 1 to 3 therefore were determined by Rankit analysis.[13, 14]

Approximately as many blood samples were drawn between 9 A.M. and 12 A.M. (n = 98) as between 1 P.M. and 3 P.M. (n = 119). When the biweekly means of plasma unconjugated, total, or immunoreactive E_3 concentrations determined in the morning were compared with the corresponding afternoon means, no significant difference could be detected by t-test analysis.

A comparison between the biweekly averages of unconjugated, total, or immunoreactive plasma E_3 in women delivering infants with birth weights between the tenth and fiftieth percentiles with the corresponding means in women delivering infants with birth weights above the fiftieth percentile is depicted in Table I. Although five of the six biweekly means of unconjugated plasma E_3 concentrations in gravidas with infants between the tenth and fiftieth birth weight percentiles were smaller than those above the fiftieth percentile, none of these differences was statistically significant (t-test). Similarly, total plasma E_3 concentrations, on the average, were higher in five out of six biweekly groups of gravidas with infant birth weights above the fiftieth percentile, but this was significant ($p < 0.05$) in only one of the biweekly groups. No

Table I. Comparison between plasma unconjugated, total, and immunoreactive estriol (E_3) in women delivering infants with birth weights between the tenth and fiftieth (A) and above the fiftieth percentile (B)

Weeks of gestation	*Unconjugated E_3*						*Total E_3*						*Immunoreactive E_3*					
	A			*B*			*A*			*B*			*A*			*B*		
	*Mean**	*S.D.**	*No.*	*Mean*	*S.D.*	*No.*	*Mean*	*S.D.*	*No.*	*Mean*	*S.D.*	*No.*	*Mean*	*S.D.*	*No.*	*Mean*	*S.D.*	*No.*
29-30	6.1	1.9	5	6.8	2.2	14	79.1	35.8	5	77.6	28.5	14	49.0	14.1	5†	34.6	8.4	14
31-32	7.6	1.2	10	7.8	2.7	20	98.1	33.4	10	116.7	45.2	20	54.6	17.5	10	48.8	16.0	20
33-34	8.9	3.1	14	10.4	4.7	26	92.0	40.5	14†	139.3	67.0	26	52.8	20.6	14	66.2	29.9	26
35-36	10.7	3.6	15	12.4	4.3	35	130.1	58.2	15	144.8	53.1	35	55.7	15.6	15	71.3	29.0	35
37-38	15.9	9.1	15	15.9	6.3	35	200.6	117.0	15	201.7	98.3	35	79.8	31.6	15	88.4	30.1	35
39-40	17.6	5.2	10	18.9	6.0	18	202.3	27.3	10	243.0	115.0	18	100.4	23.0	10	98.2	29.0	18

*Means and standard deviations (S.D.) are listed in nanograms per milliliter.
†$p < 0.05$.

Table II. Unconjugated plasma estriol concentrations (nanograms per milliliter) at various times during a 24 hour period in nine diabetic and three toxemic patients in late pregnancy

Subjects	*6* A.M.	*9* A.M.	*12* A.M.	*4* P.M.	*7* P.M.	*12* P.M.	*6* A.M.	*Average**
1	10.1 (95)†	8.1 (76)	10.9 (103)	10.6 (100)	9.7 (92)	9.5 (90)	15.1 (143)	10.6
2	16.6 (101)	12.5 (76)	15.5 (94)	17.0 (103)	18.1 (110)	16.6 (101)	18.9 (115)	16.5
3	7.7 (101)	7.5 (99)	7.3 (96)	7.8 (103)	7.9 (104)	9.8 (129)	5.5 (71)	7.6
4	16.3 (101)	13.8 (85)	16.8 (104)	19.0 (117)	16.5 (102)	15.2 (94)	15.7 (97)	16.2
5	21.4 (116)	15.7 (85)	17.8 (97)	19.1 (104)	17.9 (97)	17.1 (93)	19.9 (108)	18.4
6	8.5 (99)	7.2 (84)	7.3 (85)	8.2 (95)	8.6 (100)	9.8 (114)	10.3 (120)	8.6
7	8.4 (118)	6.5 (92)	7.3 (103)	6.9 (97)	7.2 (101)	6.6 (93)	6.6 (93)	7.1
8‡	11.3 (104)	12.8 (117)	9.1 (84)	12.5 (115)	9.9 (91)	9.5 (87)	11.3 (104)	10.9
9	16.9 (92)	18.3 (100)	16.2 (88)	18.1 (98)	18.0 (98)	18.5 (101)	22.5 (122)	18.4
10‡	11.3 (90)	11.4 (91)	11.6 (93)	12.7 (102)	13.4 (107)	13.4 (107)	14.0 (112)	12.5
11	18.0 (124)	12.5 (86)	12.3 (85)	15.4 (106)	13.0 (90)	14.3 (99)	16.1 (111)	14.5
12‡	4.5 (125)	3.7 (103)	2.3 (64)	3.6 (100)	3.9 (108)	3.8 (106)		3.6
Mean	(105.5)	(91.2)	(91.3)	(103.3)	(100.0)	(101.2)	(108.7)	
S.D.	(12.2)	(11.9)	(11.2)	(6.7)	(6.7)	(11.7)	(18.3)	

*Average of the seven observations on each subject.
†Values in parentheses represent the ratio of the observed values to the average × 100.
‡Toxemic patients.

distinct pattern was observed for immunoreactive E_3.

Assessment of possible diurnal and/or episodic variations. Unconjugated, total, and immunoreactive plasma E_3 concentrations as well as urinary E_3 and creatinine excretion were determined in 12 hospitalized women in late pregnancy over a 24 hour period. All plasma E_3 assays were carried out in duplicate on two different days. The results are

Table III. Total plasma estriol concentrations (nanograms per milliliter) at various times during a 24 hour period in nine diabetic and three toxemic patients in late pregnancy

Subjects	*6* A.M.	*9* A.M.	*12* A.M.	*4* P.M.	*7* P.M.	*12* P.M.	*6* A.M.	*Average**
1	79.2 (86†)	95.8 (104)	87.4 (95)	81.0 (88)	59.2 (64)	111.1 (120)	133.6 (144)	92.5
2	244.9 (109)	225.8 (101)	219.4 (98)	210.9 (94)	207.3 (92)	202.8 (90)	258.7 (115)	224.3
3	89.9 (84)	102.4 (95)	113.6 (106)	98.2 (91)	120.8 (113)	104.0 (97)	123.0 (115)	107.4
4	232.4 (85)	277.0 (101)	272.1 (99)	316.7 (116)	308.1 (113)	212.9 (78)	297.4 (109)	273.8
5	104.2 (88)	143.2 (121)	118.9 (100)	130.1 (110)	107.6 (91)	98.2 (83)	129.4 (109)	118.8
6	118.9 (126)	96.8 (103)	81.5 (87)	93.1 (99)	92.4 (98)	87.2 (93)	89.6 (95)	94.2
7	88.2 (139)	82.7 (130)	51.0 (80)	38.4 (61)	74.0 (117)	60.8 (96)	49.3 (78)	63.5
8‡	83.8 (92)	108.4 (119)	100.6 (111)	79.6 (88)	116.7 (128)	76.4 (84)	71.0 (78)	90.9
9	204.0 (103)	231.2 (117)	230.7 (116)	199.8 (101)	160.1 (81)	178.8 (90)	183.7 (93)	198.3
10‡	134.8 (94)	154.1 (108)	152.1 (106)	111.1 (78)	160.5 (112)	135.1 (95)	152.7 (107)	142.9
11	154.5 (124)	171.2 (138)	113.1 (91)	91.1 (73)	89.8 (72)	120.5 (97)	130.9 (105)	124.4
12‡	44.1 (113)	42.0 (107)	39.3 (100)	37.7 (96)	32.9 (84)	39.2 (100)		39.2
Mean S.D.	(103.6) (18.6)	(112.0) (13.0)	(99.1) (10.1)	(91.3) (15.4)	(97.1) (19.8)	(93.6) (10.6)	(104.4) (19.6)	

*Average of the seven observations on each subject.
†Values in parentheses represent the ratio of the observed values to the average × 100.
‡Toxemic patients.

presented in Tables II to IV and their averages are depicted in Fig. 4. The data obtained in individual patients were normalized to per cent values by calculating the ratio of the observed value to the patient's 24 hour average times 100.

For unconjugated plasma E_3, relative levels below 100 per cent were observed more frequently at 9 A.M. and 12 A.M. than at other times. The averages of the relative unconjugated plasma E_3 concentrations calculated for 9 A.M. and 12 A.M. were significantly lower ($p < 0.01$, t test) than those determined for 6 A.M., 4 P.M., 7 P.M., 12 P.M., and 6 A.M. next morning (Table II).

Relative total plasma E_3 concentrations below 100 per cent were found more frequently at 4 P.M., 7 P.M., and 12 P.M. than at 6 A.M., 9 A.M., and 6 A.M. next morning (Table III). The averages of the relative total plasma E_3 levels calculated for 12 A.M., 4 P.M., 7 P.M., and 12 P.M. were significantly lower than the 9 A.M. average with a nadir at 4 P.M. (Fig. 4). Immunoreactive plasma E_3 concentrations, as depicted in Table IV, failed to reveal any significant difference between the averages of the relative levels calculated for the various hours listed, but also exhibited a nadir at 4 P.M. (Fig. 4).

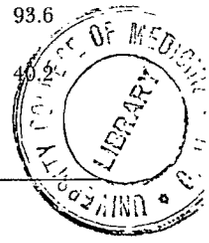
Urinary E_3 and creatinine excretion was determined in the same 12 women for six subsequent time periods during this 24 hour study. As shown in Fig. 4, E_3 excretion in milligrams per hour varied considerably. Despite large patient-to-patient variations, the average 7 P.M. to 12 P.M. E_3 excretion rate was significantly lower than that observed from 6 A.M. until 9 A.M. The E_3/creatinine ratios varied to a lesser extent than the E_3 excretion rates. A nadir was observed between 4 P.M. and 7 P.M. The 4 P.M. to 7 P.M. E_3/creatinine ratio was significantly lower than the one from 6 A.M. to 9 A.M. and from 12 P.M. to 6 A.M.

Day-to-day changes in plasma and 24-hour urinary E_3. These were determined in 14 hospitalized patients in the third trimester during observation periods ranging from 6 to 31 days. Increases in plasma and urinary E_3 above the mean of the three preceding values outnumbered and were larger than decreases as shown in Table V. The averages of the decreases observed with

Table IV. Immunoreactive plasma estriol concentrations (nanograms per milliliter) at various times during a 24 hour period in nine diabetic and three toxemic patients in late pregnancy

Subjects	*6* A.M.	*9* A.M.	*12* A.M.	*4* P.M.	*7* P.M.	*12* P.M.	*6* A.M.	*Average**
1	52.8 (97†)	59.5 (109)	51.4 (94)	40.0 (73)	37.9 (70)	61.0 (112)	78.9 (145)	54.5
2	89.7 (104)	91.4 (106)	105.9 (122)	76.5 (88)	86.3 (100)	80.1 (93)	75.9 (88)	86.5
3	43.2 (73)	44.0 (74)	60.0 (101)	62.8 (106)	76.7 (129)	67.7 (114)	60.6 (102)	59.3
4	119.6 (110)	91.7 (85)	128.6 (119)	115.3 (107)	118.6 (110)	99.8 (92)	84.8 (78)	108.3
5	84.1 (94)	106.9 (119)	96.2 (108)	101.6 (114)	94.0 (105)	79.3 (89)	64.1 (72)	89.5
6	91.2 (138)	68.5 (103)	57.5 (87)	58.6 (88)	62.2 (94)	60.3 (91)	66.1 (100)	66.3
7	52.6 (109)	56.1 (116)	39.2 (81)	35.6 (74)	55.9 (116)	51.2 (106)	47.0 (98)	48.2
8‡	63.0 (107)	61.3 (104)	56.0 (95)	62.0 (105)	79.3 (134)	49.6 (84)	41.5 (70)	59.0
9	70.9 (94)	84.3 (112)	88.7 (117)	65.8 (87)	72.9 (96)	67.0 (89)	79.9 (106)	75.6
10‡	75.7 (102)	70.3 (95)	68.1 (92)	62.3 (84)	97.4 (131)	80.7 (109)	65.2 (88)	74.2
11	106.7 (114)	104.4 (112)	91.7 (98)	81.9 (88)	75.3 (80)	98.6 (105)	96.4 (103)	93.6
12‡	49.8 (124)	36.2 (90)	48.2 (120)	41.0 (102)	32.1 (80)	34.1 (85)		40.2
Mean S.D.	(105.5) (16.3)	(102.1) (13.6)	(102.8) (14.0)	(93.0) (13.4)	(103.8) (21.2)	(97.4) (10.9)	(95.5) (21.0)	

*Average of the seven observations on each subject.
†Values in parentheses represent the ratio of the observed values to the average × 100.
‡Toxemic patients.



total and unconjugated plasma E_3 were virtually equal. Both were significantly smaller than the average of decreases in 24-hour urinary E_3. When the 24-hr urinary E_3 excretion was corrected for changes in creatinine excretion, however, average day-to-day changes were lower than those observed for plasma total and unconjugated E_3. Further analysis of the decreases, as presented in Table VI, indicates that about 2 per cent of decreases in plasma total and unconjugated E_3 exceeded 40 per cent, whereas the largest decrease observed for the urinary E_3/creatinine was below 40 per cent.

Comment

The plasma concentrations of unconjugated E_3 measured in 217 women with uncomplicated pregnancies delivering normal healthy babies agree with those reported by Den and associates,[15] Goebel and Kuss,[16] and de Hertogh and associates,[17] employing E_3-6-CMO-BSA antisera for RIA without preceding chromatography, but are larger than those published by Tulchinsky and Abraham[18] and by Cohen and Cohen,[19] who utilized celite partition chromatography prior to RIA. The biweekly averages of total plasma E_3 observed in these 217 women were lower than the weekly means reported by Nachtigall and associates[20] and Mathur and associates,[21] who used fluorimetry, but agreed better with those published by Macourt and associates,[22] who employed competitive protein-binding analysis with uterine cytosol. Unconjugated plasma E_3 averaged approximately 8 per cent of the total plasma E_3. The unconjugated-to-total E_3 ratio appeared constant during the third trimester of pregnancy. Plasma concentrations of immunoreactive E_3 comprising predominantly unconjugated E_3, E_3-3S, and E_3-3G averaged 48 per cent of total plasma E_3 throughout the third trimester. According to a previous study from this laboratory,[10] E_3, E_3-3S, and E_3-3G amount to approximately 50 per cent of total plasma E_3. Our immunoreactive plasma E_3 levels are not comparable to those measured by Podoba and associates[23] because the latter authors used the anti-E_3-16, 17-dihemisuccinate-BSA serum of Gurpide and associates[24] and reported to have measured E_3 and E_3-

Table V. Day-to-day variation in plasma and urinary estriol (E_3) in 14 women in late pregnancy hospitalized for diabetes (n = 11), toxemia (n = 1), or placenta previa (n = 2)*

	Total plasma E_3	*Unconjugated plasma E_3*	*24 hour urinary E_3*	*Urinary E_3/creatinine*
Increases	25.2 ± 22.5 n = 68	21.5 ± 16.6 n = 78	35.1 ± 33.1 n = 74	19.0 ± 15.0 n = 71
Decreases	12.6 ± 9.4 n = 59	12.4 ± 9.9 n = 49	20.6 ± 15.8 n = 53	10.9 ± 9.2 n = 56

*Expressed in per cent of the mean of three preceding determinations. Values represent averages ± 1 S.D.

Table VI. Cumulative frequency distribution of day-to-day decreases in plasma and urinary estriol (E_3) in 14 women during the third trimester of pregnancy hospitalized for diabetes (n = 11), placenta previa (n = 2), and toxemia (n = 1)

*Extent of decrease**	*Total plasma E_3*	*Unconjugated plasma E_3*	*24-hour urinary E_3*	*Urinary E_3/creatinine*
	n = 59	n = 49	n = 53	n = 56
5	20.3	30.6	17.0	32.1
10	45.7	49.0	37.8	55.3
15	74.5	69.5	39.4	73.2
20	79.6	81.7	56.7	83.9
25	89.8	87.8	64.2	89.3
30	94.9	96.0	77.4	98.2
35	94.9	96.0	81.2	98.2
40	98.3	98.0	88.7	100.
>40	100	100	100	
Largest decrease*	41.5	47.5	64.6	35.7

*In per cent of mean of three preceding determinations.

16G in ethanolic plasma extracts. Rankit analysis[13, 14] established a log-normal distribution of plasma unconjugated, total, and immunoreactive E_3 and was essential in delineating meaningful lower 95 per cent confidence limits for normal pregnancies. Differences of average unconjugated, total, and immunoreactive plasma E_3 concentrations between women with comparable gestational age who delivered infants with birth weights between the tenth and fiftieth and above the fiftieth percentiles, respectively, were not significant.

Tulchinsky and associates[5] reported to have ruled out substantial diurnal variations of unconjugated plasma E_3, but in a detailed study, Goebel and Kuss[6] demonstrated a circadian rhythm with the highest values at 8 P.M. and the lowest levels at 8 A.M., measuring unconjugated plasma E_3 in 2 hour intervals over a 24-hour period in normal late pregnancy. Our study was carried out in pregnant women hospitalized for diabetes and toxemia because it appeared clinically more important to assess the possibility and extent of diurnal and/or episodic plasma E_3 changes in high-risk pregnancies than in normal gestation. Our results document diurnal variations of unconjugated plasma E_3 with average 9 A.M. and 12 A.M. levels being approximately 10 per cent lower than average concentrations at all other hours measured, but overshadowed by sizable episodic changes.

Selinger and Levitz[2] originally reported lower total plasma E_3 concentrations at 4:30 P.M. than at 8 A.M. and 9 P.M. in normal third-trimester pregnancies. Also, Townsley and associates[3] published diurnal variations of total plasma E_3. More recently, Levitz and associates[4] presented evidence contradicting significant periodicity of total plasma E_3 levels but indicating rather large episodic fluctuations in uncomplicated pregnancies. Our results gathered from pregnant women with diabetes and toxemia are consistent with considerable episodic fluctuations in total plasma E_3 levels, but also indicate minor diurnal variations: mean 4 P.M., 7 P.M., and 12 P.M. concentrations were approximately 10 per cent lower than 6 A.M. and 9 A.M. averages.

No discernible diurnal pattern of serum immunoreactive E_3 concentrations was observed in contrast to minor diurnal variations of unconjugated and total plasma E_3. In view of substantial episodic fluctuations, the diurnal variations seem clinically irrelevant. This impression is further substantiated by the fact that no significant difference was found between averages of plasma unconjugated, total, and immunoreactive E_3 measured from 9 A.M. to 12 A.M. and 1 P.M. to 3 P.M. in groups of normal pregnant women of comparable gestational age.

A possible explanation for the decrease in unconjugated plasma E_3 during the late morning hours is the

assumption that, in response to rising maternal serum cortisol levels, fetal plasma cortisol concentrations increase due to placental transfer and reduce fetal ACTH output and production of fetal adrenal E_3 precursors.[25] The afternoon/evening decrease in total plasma E_3 might than be explained by the fact that the bulk of total plasma E_3 consists of conjugates of hepatic and enteric origin,[26] some of which reach the circulation hours after their steroid moiety was transferred across the placenta.[27] In addition, E_3-3S and E_3-3S,16G which comprise more than half of the total plasma E_3,[10] are not rapidly excreted. While unconjugated plasma E_3 levels appear to constitute an up-to-date reflection of fetoplacental E_3 production, total plasma E_3 concentrations are subject to metabolic delay, particularly by the entero-hepatic circulation.[28] The late afternoon decrease in urinary E_3 output thus may result from lower total plasma E_3 levels rather than from changes in renal clearance.

The changes in urinary E_3 excretion and the variations of urinary E_3/creatinine ratios observed over the 24-hour study period indicate that the E_3/creatinine ratio does not correct for fluctuations in E_3 excretion measured over a limited time period only. This finding is consistent with some reports,[29, 30] but in disagreement with others.[31–33] Our 5 year clinical experience with thousands of urinary E_3 and creatinine determinations permits us to conclude that the E_3/creatinine ratio is not a reliable substitute for a 24-hour urinary E_3 determination if the creatinine measured in the urine aliquot submitted for analysis comprises less than half the patient's 24-hour creatinine excretion.[34]

It is of interest to note that day-to-day changes in unconjugated plasma E_3 concentrations equaled those of total plasma E_3 and that both were considerably smaller than variations in 24-hour urinary E_3 excretion despite in-hospital urine collections. However, the smallest day-to-day changes were observed with urinary E_3/creatinine ratios on 24-hour urine collections. These data are in agreement with our conclusions from a previous larger study[12] suggesting that a fall in urinary E_3 of more than 35 per cent may be regarded as "significant" if it is accompanied by a comparable decrease in E_3/creatinine ratio. Based on limited data from 14 patients totaling 127 observations of day-to-day changes in this study, a fall in plasma unconjugated and/or total E_3 may have to exceed some 40 to 45 per cent of the mean of the three preceding determinations in order to have less than a 1 per cent likelihood of representing a change which is unrelated to reduced fetoplacental E_3 production secondary to fetal distress. Further long-term experiments in high-risk pregnancies are necessary to establish whether fetal distress drops plasma unconjugated and/ total E_3 in excess of 40 to 45 per cent and whether or not recognition of such a decrease within 24 hours would leave sufficient time for successful obstetric intervention.

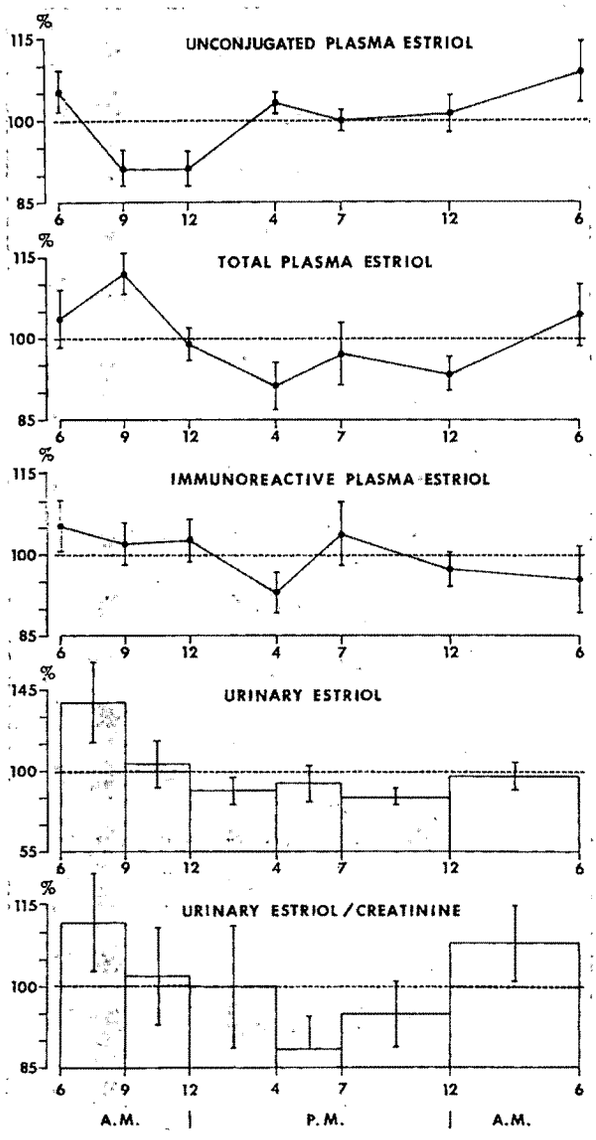


Fig. 4. Averages and standard errors of relative unconjugated, total, and immunoreactive plasma E_3 concentrations and of urinary estriol and estriol/creatinine excretion rates at various times in a 24 hour period. The data obtained in 12 individual patients were normalized to per cent values by calculating the ratio of the observed value to the patient's 24 hour average × 100.

The authors are indebted to Mrs. A. G. Freeman, R. N., B. S., for collecting blood specimens from normal pregnancies, to the Nursing Staff from the high-risk antepartum ward of the LAC-USC Women's Hospital for their kind cooperation, and to Miss Y. Livett for expert technical assistance.

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The reasons for acceptance or rejection of an oral contraceptive are many. Some are hers and some are yours.

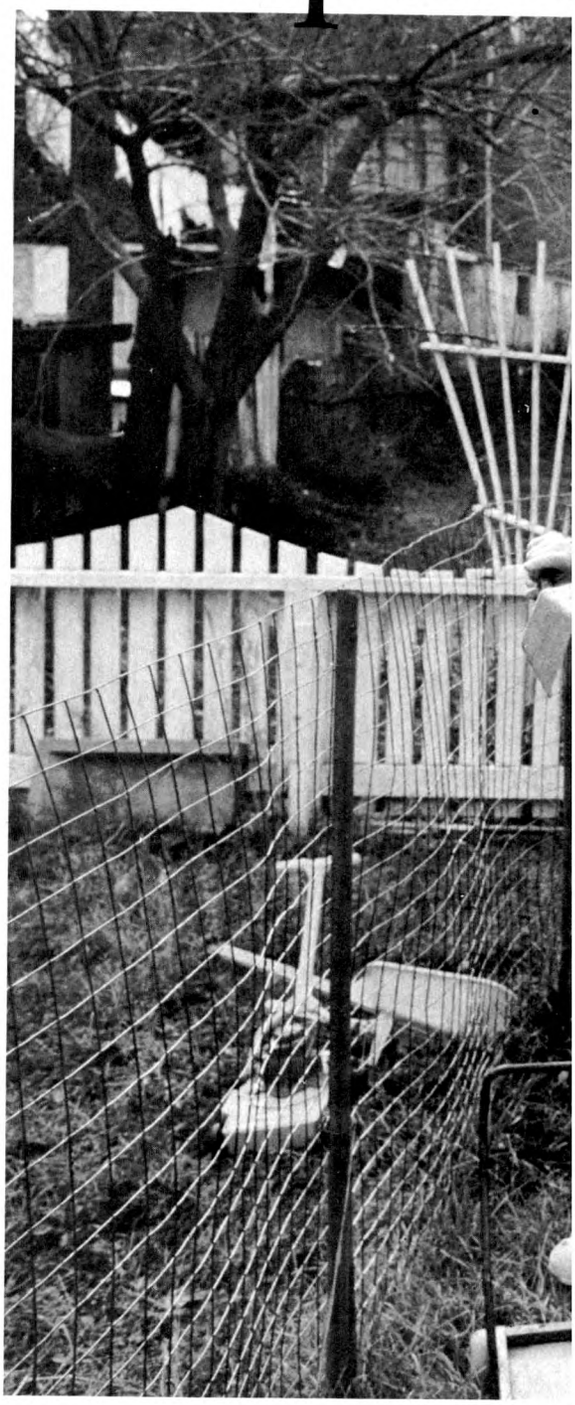
In the main, her reasons for rejection, often highly subjective, are based on side effects—and side effects are only partially predictable from history and examination.

The ultimate test, then, of oral contraceptive acceptability must be what happens when she takes it since both your judgment and hers will be based on...

Real Life Experience

From real life...

A Woman satisfied her oral contraceptive



One important reason for patient satisfaction: Usually no G.I. discomfort

ZORANE 1/50 is a highly effective and generally well-tolerated oral contraceptive—providing the estrogen content and progestin:estrogen ratio that appears to be highly acceptable to the majority of women.

From clinical studies: In a study population of 1156 women, 25,983 cycles, the incidence of nausea and vomiting was 3.3%. Actual patient dropout rate* due to nausea and vomiting was 2.9% (33 subjects.) Overall patient dropout rate was 14.4% due to all adverse reactions. Serious reactions, as well as minor adverse reactions, have been reported following the use of all oral contraceptives. Before prescribing, the complete product information should be consulted for *special note, contraindications, warnings, precautions* and *adverse reactions*. Thereafter, the physician should remain alert to the earliest manifestations of any serious disorder. Should any occur or be suspected, the oral contraceptive should be immediately discontinued.

The woman profiled here is an actual ZORANE 1/50 user. However, not all women can be expected to have the same tolerance for side effects, should any occur. (Data on file, Lederle Laboratories)

*Dropout rates are based on total patient population (1,156); incidence reflects only those patients who completed one or more cycles (956).

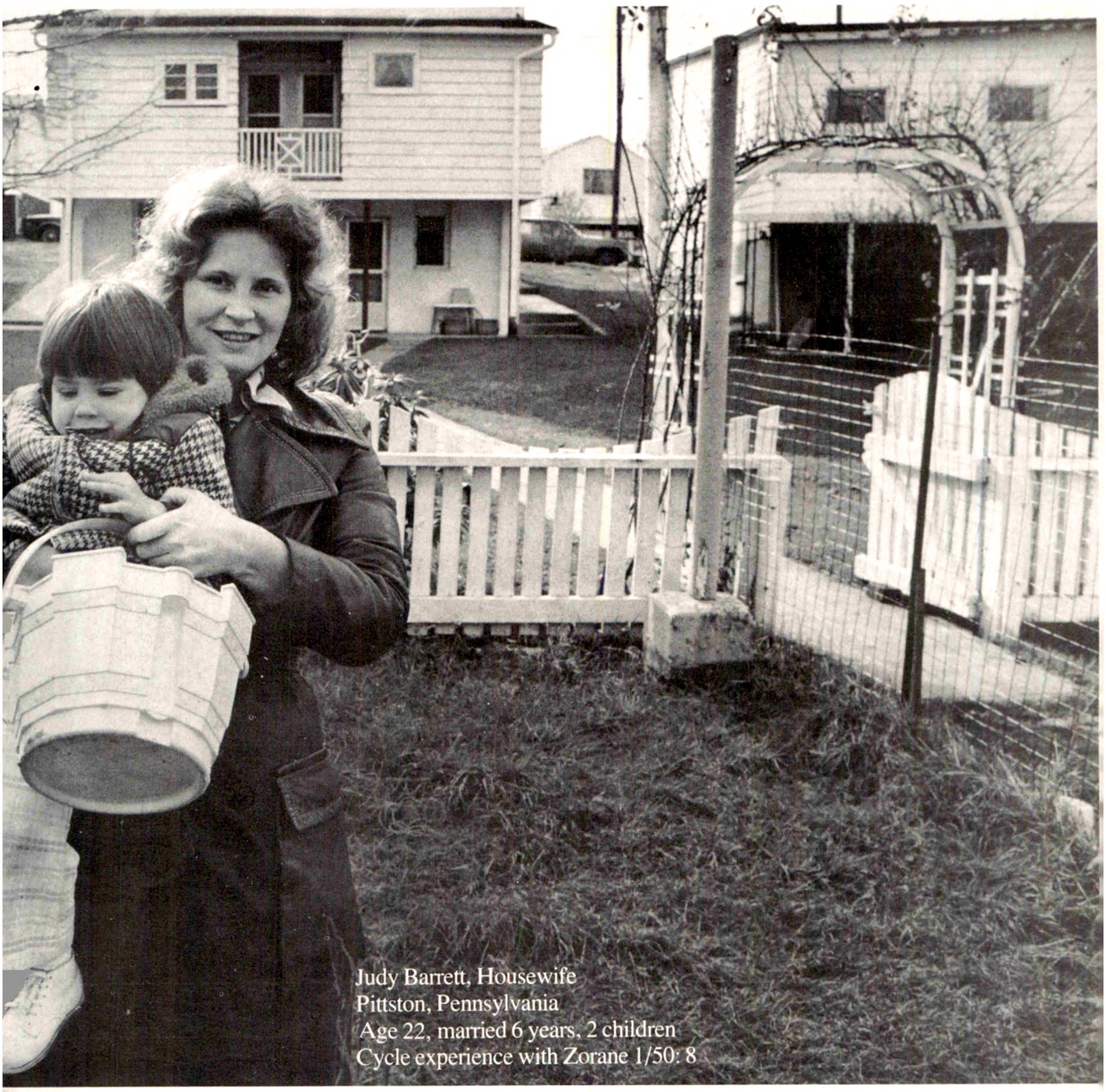
Zorane® 1/50

Norethindrone Acetate, 1 mg and Ethinyl Estradiol, 50 mcg
Oral Contraceptive Tablets

Progestin/Estrogen content balanced fo

See last page of this advertisement for brief summary of prescribing information.

with



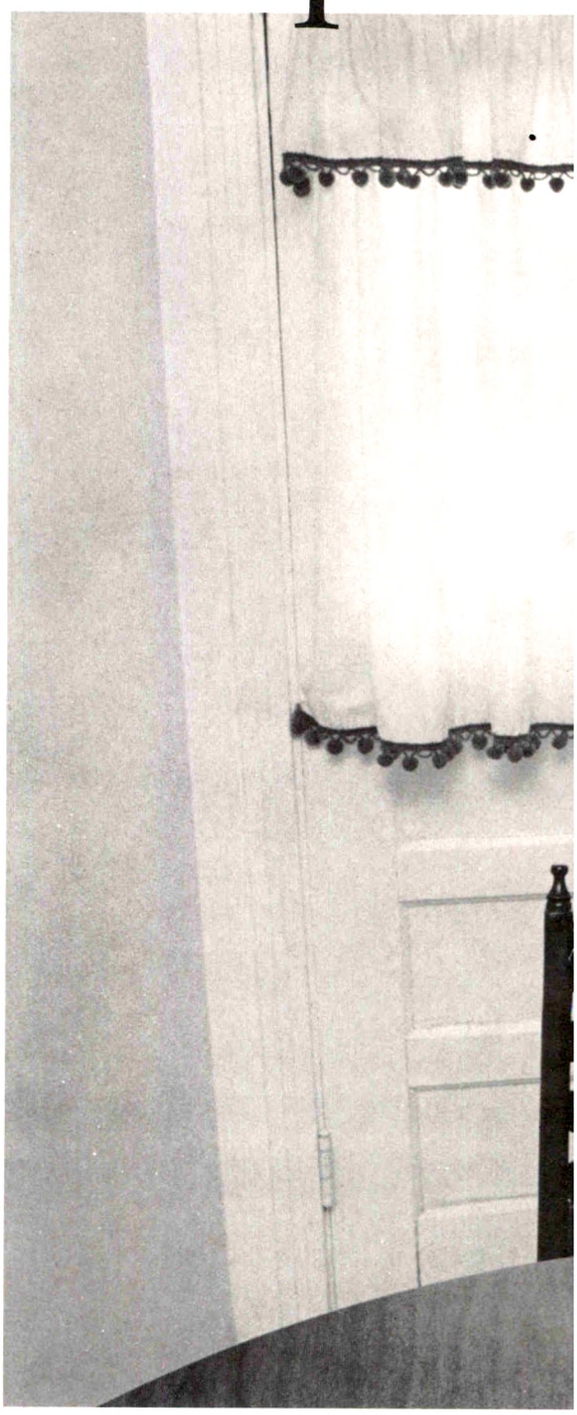
Judy Barrett, Housewife
Pittston, Pennsylvania
Age 22, married 6 years, 2 children
Cycle experience with Zorane 1/50: 8

he majority

"I don't seem to gain much weight with the pill and I don't get sick. I take it at night just before I go to bed... then I sleep right through. And I don't get sick."

From real life...

A Woman satisfiec her oral contraceptive



Among the reasons patients remain satisfied: Withdrawal bleeding is usually regular and there is usually no undue discomfort

ZORANE 1/50 is a highly effective and generally well-tolerated oral contraceptive—providing the estrogen content and progestin:estrogen ratio that appears to be highly acceptable to the majority of women.

From clinical studies: In a study population of 1156 women, 25,983 cycles, the incidence was 2.2% for amenorrhea and 2.0% for dysmenorrhea. Actual patient dropout rate* was 1.1% (13 subjects) for amenorrhea and 0.2% (2 subjects) for dysmenorrhea. Overall patient dropout rate was 14.4% due to all adverse reactions. Serious reactions, as well as minor adverse reactions, have been reported following the use of all oral contraceptives. Before prescribing, the complete product information should be consulted for *special note, contraindications, warnings, precautions* and *adverse reactions*. Thereafter, the physician should remain alert to the earliest manifestations of any serious disorder. Should any occur or be suspected, the oral contraceptive should be immediately discontinued.

The woman profiled here is an actual ZORANE 1/50 user. However, not all women can be expected to have the same tolerance for side effects, should any occur. (Data on file, Lederle Laboratories)

*Dropout rates are based on total patient population (1,156); incidence reflects only those patients who completed one or more cycles (956).

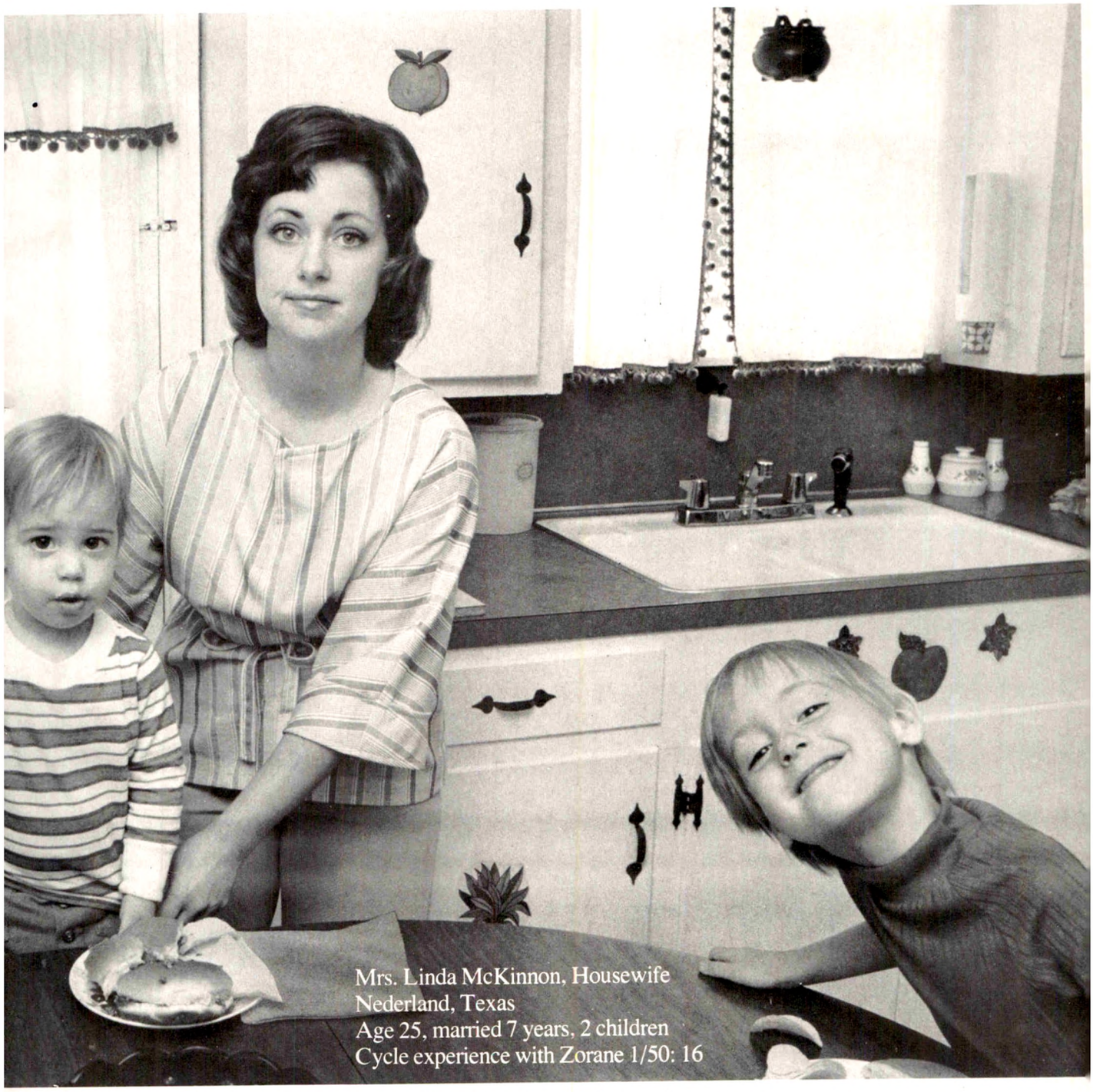
Zorane® 1/50

Norethindrone Acetate, 1 mg and Ethinyl Estradiol, 50 mcg
Oral Contraceptive Tablets

Progestin/Estrogen content balanced fo

See last page of this advertisement for brief summary of prescribing information.

with



Mrs. Linda McKinnon, Housewife
Nederland, Texas
Age 25, married 7 years, 2 children
Cycle experience with Zorane 1/50: 16

he majority

"You always know when you're going to start... I love knowing that... you can always plan... I think it's great. And, not having any cramps, too, because I used to have cramps."

From real life...

A Woman satisfiec her oral contraceptive



One reason for long-term patient satisfaction: Weight gain usually not a problem

ZORANE 1/50 is a highly effective and generally well-tolerated oral contraceptive—providing the estrogen content and progestin:estrogen ratio that appears to be highly acceptable to the majority of women.

From clinical studies: In a study population of 1156 women, 25,983 cycles, the incidence of weight gain was 0.4%. Actual patient dropout rate* due to weight gain was 2.0% (23 subjects). Overall patient dropout rate was 14.4% due to all adverse reactions. Serious reactions, as well as minor adverse reactions, have been reported following the use of all oral contraceptives. Before, prescribing, the complete product information should be consulted for *special note, contraindications, warnings, precautions* and *adverse reactions*. Thereafter, the physician should remain alert to the earliest manifestations of any serious disorder. Should any occur or be suspected, the oral contraceptive should be immediately discontinued.

The woman profiled here is an actual ZORANE 1/50 user. However, not all women can be expected to have the same tolerance for side effects, should any occur. (Data on file, Lederle Laboratories)

*Dropout rates are based on total patient population (1,156); incidence reflects only those patients who completed one or more cycles (956).

Zorane® 1/50

♀ Norethindrone Acetate, 1 mg and Ethinyl Estradiol, 50 mcg
Oral Contraceptive Tablets

Progestin/Estrogen content balanced fo

See last page of this advertisement for brief summary of prescribing information.

with



Alice Eichenberger, High School
Business Teacher, Louisville, Kentucky
Age 28, married 7 years, no children
Cycle experience with Zorane 1/50: 13

the majority

"I've fluctuated between 115 and 120...and have had no appreciable weight gain...since I've been on Zorane."

Zorane® 1/50

Progestin/Estrogen content balanced for the majority

♀ Norethindrone Acetate, 1 mg and Ethinyl Estradiol, 50 mcg

Oral Contraceptive Tablets

Also available from Lederle

Zorane 1.5/30 ♀ Norethindrone Acetate, 1.5 mg. and Ethinyl Estradiol, 30 mcg.

Zorane 1/20 ♀ Norethindrone Acetate, 1 mg. and Ethinyl Estradiol, 20 mcg.

Special Note: Oral contraceptives have been marketed in the United States since 1960. Reported pregnancy rates vary from product to product. The effectiveness of sequential products appears to be somewhat lower than that of combination products. Both types provide almost completely effective contraception. The dropout rate for medical reasons, as observed in clinical trials, with ZORANE 1.5/30 appears to be the same as that of higher dose combination products. The dropout rate due to irregular bleeding was somewhat higher, but dropouts were equally split between irregular bleeding and other medical reasons attributable to the drug. (ZORANE 1/20 only: The effectiveness is somewhat lower than that of the higher dose combination products. Both provide almost completely effective contraception. Dropout rate for medical reasons appears to be somewhat higher than with higher dose combination products, due particularly to irregular bleeding.) An increased risk of thromboembolic disease associated with use of hormonal contraceptives has now been shown in studies conducted in both Great Britain and the United States. Other risks, such as those of elevated blood pressure, liver disease, reduced tolerance to carbohydrates, have not been quantitated with precision. Long-term administration of both natural and synthetic estrogens in subprimate animal species in multiples of the human dose increases the frequency for some animal carcinomas. These data cannot be transposed directly to man. Possible carcinogenicity due to the estrogens can neither be affirmed nor refuted at this time. Close clinical surveillance of all women taking oral contraceptives must be continued.

Indications: For control of conception.

Contraindications: Thrombophlebitis, thromboembolic disorders, cerebral apoplexy, or a past history of these conditions; markedly impaired liver function; known or suspected carcinoma of the breast; known or suspected estrogen-dependent neoplasia; undiagnosed abnormal genital bleeding; pregnancy.

Warnings: If early signs of thrombotic disorders (thrombophlebitis, cerebrovascular disorders, pulmonary embolism, retinal thrombosis) occur or are suspected, discontinue use of drug immediately. (Studies indicated a higher risk of these conditions with use of these drugs, not persisting after drug stoppage and not enhanced by long-term use.[1-4]) If sudden partial or complete loss of vision occurs, or if there is sudden onset of proptosis, diplopia or migraine; or if examination reveals papilledema or retinal vascular lesions, discontinue medication. Safety of use in pregnancy has not been established, therefore rule out pregnancy in any patient who has missed two consecutive periods before continuing contraceptive regimen; if prescribed schedule has not been adhered to, consider possibility of pregnancy with first missed period. A small fraction of hormonal agents has been identified in the milk of mothers receiving these drugs; long-range effect to the nursing infant has not yet been determined.

Precautions: Pretreatment and periodic physical examinations of breasts and pelvic organs should include Papanicolaou smear. (Estrogens have produced tumors, some malignant, in 5 species of subprimate animals.) If endocrine and liver function tests are abnormal in patients taking these drugs, repeat tests after drug withdrawal for 2 months. With use of these drugs, pre-existing uterine fibromyomata may increase in size; and some conditions (*i.e.*, epilepsy, migraine, asthma, cardiac or renal dysfunction) may be influenced by fluid retention; observe carefully for such. With use of ZORANE 1.5/30 or ZORANE 1/20, alteration in menstrual patterns may be induced. Since the amount and duration of flow and cycle length may vary, this must be considered in attempting to determine if cause of bleeding is organic. (ZORANE 1/50 only: In breakthrough bleeding or all cases of irregular bleeding per vaginam, keep in mind nonfunctional causes and take adequate diagnostic measures.) With history of psychic depression, discontinue drug if depression recurs to a serious degree. Possible influence of prolonged ZORANE therapy on pituitary, ovarian, adrenal, hepatic or uterine function awaits further study. A decrease in glucose tolerance has been seen in some patients on oral contraceptives; carefully observe diabetics on such therapy. Patient age is no absolute limiting factor, although these drugs may mask onset of climacteric. The pathologist should be advised of ZORANE therapy when relevant specimens are submitted. Susceptible women may have an increase in blood pressure following use of contraceptive steroids.

Adverse Reactions Observed in Patients Receiving Contraceptives: Statistically significant association demonstrated between use of oral contraceptives and: thrombophlebitis, pulmonary embolism, cerebral thrombosis. Suggestive evidence, neither confirmed nor refuted, between use and neuro-ocular lesions (*e.g.*, retinal thrombosis, optic neuritis). Known to occur: nausea, vomiting, G.I. symptoms (abdominal cramps, bloating), breakthrough bleeding, spotting, change in menstrual flow, amenorrhea during and after treatment, edema, chloasma or melasma, breast changes (tenderness, enlargement, secretion), weight change (increase or decrease), changes in cervical erosion and secretions, suppression of lactation when given immediately post-partum, cholestatic jaundice, migraine, allergic rash, rise in blood pressure in susceptible women, mental depression. Reported association with use, neither confirmed nor refuted: anovulation post treatment, premenstrual-like syndrome, changes in libido, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, fatigue, backache, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, itching. *May Alter Laboratory Results in:* hepatic function (increased sulfobromophthalein retention and other tests), coagulation tests (increase in prothrombin, Factors VII, VIII, IX, X), thyroid function (increase in PBI_1, and butanol extractable protein bound iodine, and decrease in T^3 uptake values), metyrapone test, pregnanediol determination.

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GYNECOLOGY

Treatment of cervical carcinoma

CLAYTON T. BEECHAM, M.D.
DAVID D. BEILER, M.D.
Danville, Pennsylvania

A comparison of cervical carcinoma survival data is made between a large specialized cancer treatment center, M. D. Anderson, and a general regional medical center with 15,000 inpatient admissions and over 250,000 outpatient visits per year. End results are identical and did not depend on the percentage of time spent by the gynecologists or radiation therapists on problems of pelvic cancer.

AS REGIONALIZATION of cancer care receives increasing emphasis, certain prevailing concepts need validation or possible challenge. Among the criteria considered applicable to therapeutic cancer centers, where the best possible care is given, are (1) number of new cases treated per year and (2) clinical time gynecologists devote strictly to cancer work.

A comparative study of cancer survival figures should help reconcile the issue of optimum results and its relationship to the number of new cases treated per year. The other point is more subtle and cannot be directly measured; however, examination of these important issues in the context of survival figures is relevant.

Material and methods

In 1965 the authors[1] established a protocol for the treatment of invasive cervical carcinoma. According to plan, once the diagnosis is established, the care of patients is a team effort by gynecologists and therapeutic radiologists. They work together every step of the way. Administration of radiation follows the standards set by Fletcher[2] and, while some flexibility is permitted, a therapeutic design is carefully followed. If alterations seem indicated they are made jointly by gynecologists and radiologists. Decisions for the use of radical surgery as either primary or secondary component of the treatment plan are arrived at the same way. Taylor's[3] suggestion of "a mutually advantageous system of cooperation" is operational.

Staging of the disease is done in a conventional manner and always in consultation with at least one other therapeutic radiologist and two other gynecologists. Stage IV lesions are clearly established with positive biopsies from either the bladder or rectum or both.

Into this therapeutic environment 384 cases of cervical carcinoma have come during the period 1965-1972 (Table I). To answer our questions we have stud-

Table I. Carcinoma of the cervix (1965-1972)

	(No.)
Stage 0	137
Stages I to IV	200
Stage V (incomplete treatment elsewhere)	47
Total	384

From the Departments of Gynecology-Obstetrics and Therapeutic Radiology, Geisinger Medical Center.

Presented at the Central Pennsylvania Gynecological Society Meeting, Danville, Pennsylvania, June 14, 1974.

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Reprint requests: Dr. Clayton T. Beecham, Geisinger Medical Center, Danville, Pennsylvania 17821.

Table II. Stage I carcinoma of the cervix (1965-1972)*

1	2	3	4	5	6	7	8	9	10	S. E.
1	50	0	0	0	49.5	0	1.0	1.0	1.0	0
2	49	0	1	7	45	0	1.0	1.0	0	0
3	41	1	1	10	35	0.29	0.971	0.971	1.0	0.026
4	28	0	0	6	25	0	1.0	0.971	0	0
5	22	0	0	0	22	0	1.0	0.971	0	0

*Legends:
Column 1. Years of observation; $\times$ to $\times + 1$.
Column 2. Alive at beginning of interval; l_x.
Column 3. Died during interval; d_x.
Column 4. Lost to follow-up during interval; u_x.
Column 5. Withdrawn alive during interval; w_x.
Column 6. Effective number exposed to risk of dying; l'_x.
Column 7. Proportion dying; q_x.
Column 8. Proportion surviving; p_x.
Column 9. Cumulative proportion surviving from treatment through end of interval: P_x.
Column 10. Intercurrent deaths.
S. E., standard error.

Table III. Stage II carcinoma of the cervix (1965-1972)*

1	2	3	4	5	6	7	8	9	10	S. E.
1	84	7	0	0	83	0.084	0.916	0.916	2	0.030
2	75	2	0	4	72	0.028	0.972	0.890	2	0.019
3	67	1	1	9	62	0.016	0.984	0.876	0	0.015
4	56	2	1	14	48	0.062	0.958	0.839	1	0.027
5	38	0	1	8	33.5	0.000	1.000	0.839	0	0.000

*Legends:
Column 1. Years of observation; $\times$ to $\times + 1$.
Column 2. Alive at beginning of interval; l_x.
Column 3. Died during interval; d_x.
Column 4. Lost to follow-up during interval; u_x.
Column 5. Withdrawn alive during interval; w_x.
Column 6. Effective number exposed to risk of dying; l'_x.
Column 7. Proportion dying; q_x.
Column 8. Proportion surviving; p_x.
Column 9. Cumulative proportion surviving from treatment through end of interval; P_x.
Column 10. Intercurrent deaths.
S. E., standard error.

ied the 200 Stages I to IV patients treated entirely at Geisinger Medical Center—52 per cent of our cervical malignancy problem. These have been uniformly managed in accordance with our protocol for invasive cervical carcinoma.

The data have been collated and analyzed in accordance with the modified life table method for cancer staging and end-result reporting. In brief, "this method utilizes all survival information accumulated up to the closing date of the study and describes the manner in which the patient group was depleted during the total period of observation"[4] (see legends for Tables II to IV).

Table IV. Stage III carcinoma of the cervix (1965-1972)*

1	2	3	4	5	6	7	8	9	10	S. E.
1	53	11	2	0	51	0.216	0.784	0.784	2	0.058
2	38	11	0	2	36.5	0.301	0.699	0.548	1	0.074
3	24	3	1	4	21.5	0.140	0.860	0.471	0	0.071
4	16	0	0	3	14.5	0.0	1.000	0.471	0	0.000
5	13	1	0	0	13.6	.077	0.923	0.435	0	0.074

*Legends:
Column 1. Years of observation; $\times$ to $\times + 1$.
Column 2. Alive at beginning of interval; l_x.
Column 3. Died during interval; d_x.
Column 4. Lost to follow-up during interval; u_x.
Column 5. Withdrawn alive during interval; w_x.
Column 6. Effective number exposed to risk of dying; l'_x.
Column 7. Proportion dying; q_x.
Column 8. Proportion surviving; p_x.
Column 9. Cumulative proportion surviving from treatment through end of interval; P_x.
Column 10. Intercurrent deaths.
S. E., standard error.

Table V. Stage IV carcinoma of the cervix (1965-1972)*

1	2	3	4	5	6	7	8	9	10	S. E.
1	13	5	1	0	12	0.417	0.583	0.583	1	0.137
2	6	3	0	0	5.5	0.545	0.455	0.285	1	0.203
3	2	2	0	0	2	1.000	0.0	0.0	0	0
4	0	0	0	0	0	0	1.0	0	0	0
5	0	0	0	0	0	0	1.0	0	0	0

*Legends:
Column 1. Years of observation; $\times$ to $\times + 1$.
Column 2. Alive at beginning of interval; l_x.
Column 3. Died during interval; d_x.
Column 4. Lost to follow-up during interval; u_x.
Column 5. Withdrawn alive during interval; w_x.
Column 6. Effective number exposed to risk of dying; l'_x.
Column 7. Proportion dying; q_x.
Column 8. Proportion surviving; p_x.
Column 9. Cumulative proportion surviving treatment through end of interval; P_x.
Column 10. Intercurrent deaths.
S. E., standard error.

Results

The results for the series are given according to stage of disease in Tables II to V and for all stages, as a group, in Table VI. Summarizing the actuarial calculations, our 5 year survival rate is shown in Table VII. Computer-controlled graphs (Figs. 1 to 3) lend additional visibility to the data.* M. D. Anderson's results are compared with ours in Table VIII.

*Statistical analysis by D. J. Wright, Ph.D.

Table VI. All stages of carcinoma of the cervix (1965-1972)*

1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*S. E.*
1	200	23	3	0	195.5	0.118	0.882	0.882	6	0.023
2	168	16	1	13	159	0.101	0.899	0.794	4	0.023
3	134	7	3	23	120.5	0.058	0.942	0.747	1	0.020
4	100	2	1	23	87.5	0.023	0.977	0.730	1	0.015
5	73	1	1	8	68.5	0.015	0.985	0.720	0	0.014

*Legends:
Column 1. Years of observation; $\times$ to $\times + 1$.
Column 2. Alive at beginning of interval; l_x.
Column 3. Died during interval; d_x.
Column 4. Lost to follow-up during interval; u_x.
Column 5. Withdrawn alive during interval; w_x.
Column 6. Effective number exposed to risk of dying; l'_x.
Column 7. Proportion dying; q_x.
Column 8. Proportion surviving; p_x.
Column 9. Cumulative proportion surviving from treatment through end of interval; P_x.
Column 10. Intercurrent deaths.
S.E., standard error.

Table VII. Five-year survival summation

	(%)
Stage I	97.1
Stage II	83.9
Stage III	43.5
Stage IV	0
All stages	72.0

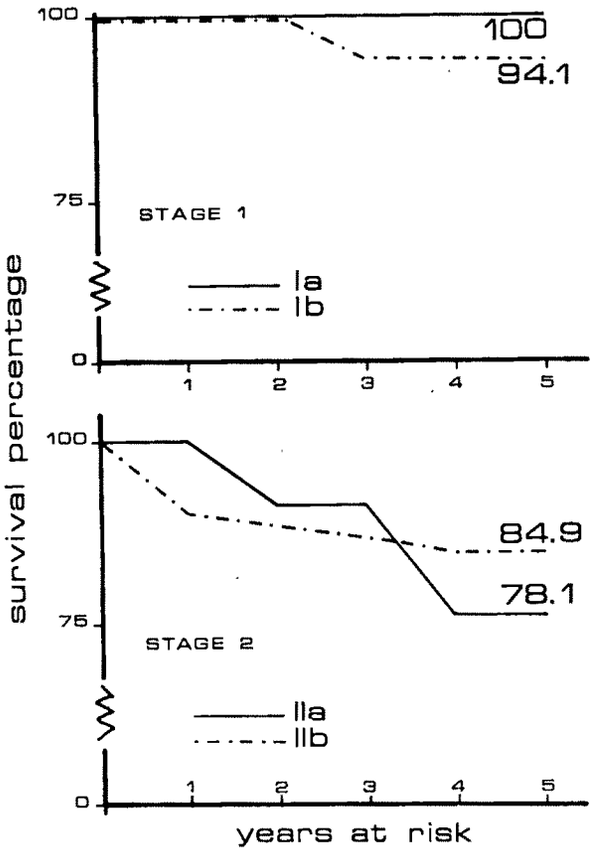


Fig. 1. Carcinoma of the cervix, Stages I and II (1965-1972).

Comment

Symmonds,[5] in his paper entitled, "Where gynecologic cancer should be treated." dealt with the issues under discussion here. He summed up by stating that optimum results are achieved where there exists a "desirable combination of adequate facilities, cancer volume, and talented personnel." These characteristics—the mark of an acceptable cancer treatment center—leave little room for argument. Can we be more specific and examine each of Symmonds' categories more closely?

First, there is essentially no end-result difference when comparing the country's largest gynecologic cancer service, M. D. Anderson, and one of the nation's smaller, Geisinger Medical Center. Whether they are representative centers for large or small case loads cannot be categorically stated. From our work it is plain that a smaller institution can duplicate the survival rate of the larger. However, we are left with no definition of "small" or what constitutes an "adequate volume" of cervical cancer cases. The *Manual of Standards*[6] suggests "a minimum of 25 new cases of invasive cervical carcinoma."

The Wainright Tumor Clinic Association's 1967 survey of results in Pennsylvania Hospitals with tumor registries did not show a positive correlation between high case load and good results. In fact, an institution with 32 new cervical cases per year (the highest) reported an over-all 5 year survival rate of 18.6 per cent.

Second, "adequate facilities," as mentioned by Symmonds, must be taken for granted. To adequately care for women with cervical carcinoma, megavoltage equipment under the surveillance of a radiation physicist, backed up by computerized calculations, and operated by therapeutic radiologists is so basic to the issue that alternatives are truly hazardous.

Third, Symmonds' "talented personnel" requirement for involvement in successful oncologic treatment is an acceptable premise. Both gynecologist and radiologist must possess special oncologic knowledge while their experience and skills are enhanced by a full range of clinical activity—not just malignancy problems for the gynecologist nor only pelvic neoplasia for the therapeutic radiologist.

Our results show that both radiologists and gyne-

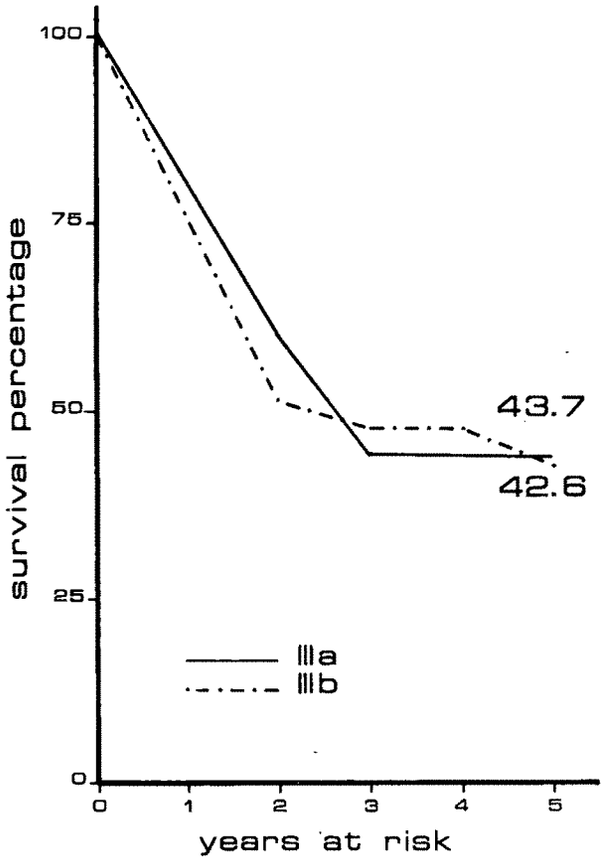


Fig. 2. Carcinoma of the cervix, Stage III (1965-1972).

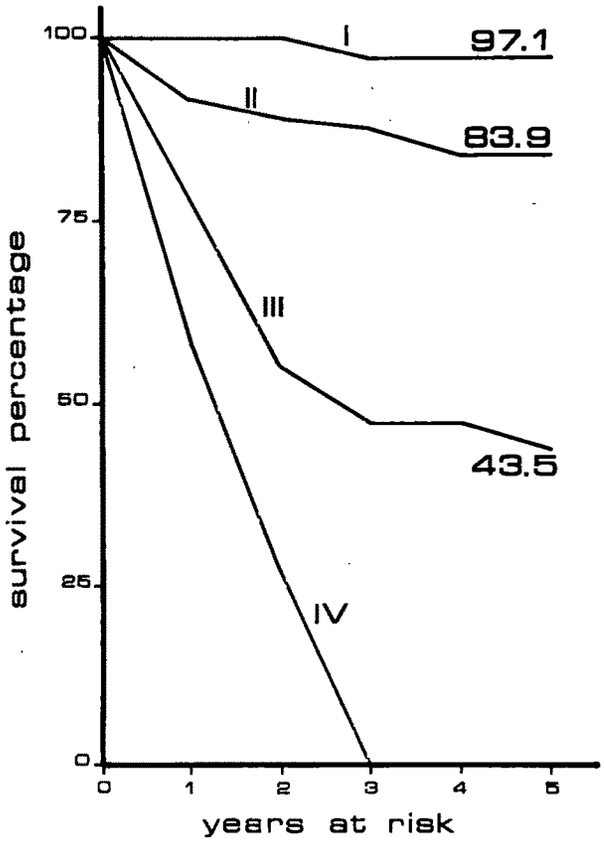


Fig. 3. Carcinoma of the cervix, Stages I to IV (1965-1972).

Table VIII. Comparative five-year survival rates for cervical carcinoma

	M. D. Anderson Hospital, 1,705 patients (%)	*Geisinger Medical Center, 200 patients (%)*
Stage I	91.5	97.1
Stage IIa	83.5	78.1
Stage IIb	66.6	84.9
Stage IIIa	45.0	42.6
Stage IIIb	36.0	43.7
Stage IV	14.0	0

cologists possess optimum clinical expertise for dealing with cervical cancer. They do not need to spend their working life in any set percentage of pelvic oncology activity but rather maintain a full range of therapeutic action in their respective fields. This, and not percentages, is a more logical basis for achieving and maintaining diagnostic and therapeutic skills.

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Clear-cell adenocarcinoma of the vagina and cervix: Survival data

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Survival data on 36 patients with clear-cell adenocarcinomas are reported: seven with vaginal lesions and 29 with cervical lesions. Although the tumor responds to present treatment modalities, it tends to be associated with early lymph node involvement, thus making early diagnosis essential. Radiation as a primary therapeutic measure has often been followed by late recurrence, though many patients with these tumors were treated by older, low-energy modalities.

SEVERAL RECENT papers have reported an association between maternal synthetic estrogen treatment and the development of clear-cell adenocarcinoma of the vagina and cervix in young women.[1–5] Although the risk to those exposed in utero may be less than was earlier anticipated, about 200 such cases are now listed in the national registry, and the specter of cancer remains over all young women whose mothers were treated with diethylstilbestrol.[6]

Because the association of cancer and diethylstilbestrol has been reported only recently, no long-term survival results are available from the tumor registry, although a preliminary report has been published.[7] We thought it would be of benefit to report the survival statistics for our patients who had clear-cell cancer. Although most of these patients were older and were without a positive maternal hormone history, our experience may help in planning treatment for younger patients exposed to maternal diethylstilbestrol who have clear-cell tumors.

Materials and methods

A total of 675 patients with primary cervical adenocarcinoma were seen at the Mayo Clinic from 1910 through 1972. A review of slides from these cases revealed 29 in which the diagnosis of clear-cell (mesonephric) adenocarcinoma could be made. Likewise, 20 cases of primary vaginal adenocarcinoma have been diagnosed between 1930 and April 1, 1974. On review, seven of the 20 were clear-cell adenocarcinomas. Maternal history was obtained for patients with either vaginal or cervical malignancy of this type who were born since 1939, and this has been reported in detail elsewhere.[8] Of 10 patients born since the synthesis of diethylstilbestrol and previously reported, four patients (one with vaginal and three with cervical cancers) had had associated maternal synthetic estrogen treatment. Since the initial report, two additional patients with vaginal clear-cell cancer have been seen and both of these had associated maternal diethylstilbestrol treatment.

We used FIGO standards to establish the primary site of the tumor as well as its clinical stage. When no stage was assigned on initial work-up, staging was done retrospectively from the data in the records. Where differences between the clinical stage and the pathologic extent of the tumor were found, these are noted in the text.

Findings

Vaginal tumors. Seven patients with primary vaginal clear-cell adenocarcinomas were treated at the Mayo Clinic between 1930 and April 1, 1974 (Table I). Surgery was the treatment of choice for patients who

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Table I. Survival among seven patients who had clear-cell adenocarcinoma of the vagina (Mayo Clinic, 1930-1974)

Stage	*Age at diagnosis (yr.)*	*Treatment*	*Lymph nodes (at surgery)*	*Status*
I	10*	Radical hysterectomy and vaginectomy	Neg.	Alive, 7 yr.
	18	Radical hysterectomy and vaginectomy	Neg.	Alive, 6 yr. 6 mo.
	20*	Radical hysterectomy and vaginectomy	Neg.	Alive, 10 mo.
	20*	Radical hysterectomy and vaginectomy	Neg.	Alive, 4 mo.
	34	Radical hysterectomy and vaginectomy	Neg.	Alive, 4 yr. 3 mo.
II	49	Radiation (250 kV.) (exenteration 2 yr. later for recurrence)	— (Neg.)	Alive, 8 yr. 3 mo. (alive, 6 yr. 2 mo.)
IV	57	Biopsy only	—	Dead (9 mo.)†

*Associated maternal diethylstilbestrol treatment.
†Survival from diagnosis to death from disease.

Table II. Staging in 29 cases of clear-cell adenocarcinoma of cervix (Mayo Clinic, 1910-1972)

Stage	*No. of patients*	
I	5	
I-A		2
I-B		2
Indeterminate		1
II	11	
II-A		7
II-B		3
Indeterminate		1
III	9	
IV	4	
Total	29	

had Stage I tumors. One patient (Stage II lesion) required total exenteration 24 months after the completion of radiation therapy but is now tumor-free at 74 months of follow-up. Three of the seven patients are 5 year survivors and three others are alive and well 4, 10, and 51 months after operation. One patient with Stage IV disease died 9 months after diagnosis.

Cervical tumors. Sixteen patients had Stage I or II lesions and 13 had Stage III or IV lesions (Table II).

Although all five patients with Stage I disease had lesions confined to the cervix, three had clinically underdiagnosed lesions at surgery (Table III). In one patient the tumor extended to the vaginal wall; in another the iliac lymph nodes were positive. The third patient had had a malignant polyp removed by her local physician. She was referred to the Mayo Clinic where, at surgery, she had no residual tumor in the cervix or uterus but had several positive aortic nodes. This patient had a positive maternal history for diethylstilbestrol.

Eight of the 11 patients with Stage II lesions underwent exploratory laparotomy and definitive surgery (Table IV). Of the eight, three had positive lymph nodes and two of the three died of recurrent disease. Three patients received primary irradiation, two after biopsy only and the third was considered inoperable at exploration. All three patients treated with irradiation have died of disease, although two survived 5 years.

All nine patients with Stage III lesions were treated with primary irradiation (Table V). All nine had recurrent disease and seven have died. Three patients underwent exenteration for recurrence after irradiation and two of the three are alive more than 5 years after exenteration.

Three patients with Stage IV disease were seen in a preterminal state, and all three lived less than 1 month. A fourth patient received palliative irradiation and died 17 months after diagnosis.

Delay in therapy. Twenty-two patients had delay in diagnosis from the onset of irregular vaginal bleeding, which was the only presenting symptom (Table VI). The seven patients who had no delay were asymptomatic and the lesions were diagnosed by routine examination. For another seven patients the delay interval was not recorded. Two patients with Stage III cervical tumors were asymptomatic; for an additional three with Stage III lesions the delay to diagnosis was 6 months or less.

Tumors associated with maternal diethylstilbestrol. Three patients with vaginal and three (possibly four) with cervical tumors had had associated maternal diethylstilbestrol. The cervical tumors (with the exception of the cervical polyp) could represent extension of a primary vaginal tumor but would be considered "cervical" tumors under FIGO standards.

All seven patients were treated with radical hysterectomy; postoperative irradiation was given to the two patients who had positive lymph nodes (Table VII). In

Table III. Survival among five patients who had clinical Stage I clear-cell adenocarcinoma of the cervix (Mayo Clinic, 1910-1972)

Stage	*Age at diagnosis (yr.)*	*Treatment*	*Lymph nodes*	*Status*
I-A	7	Local excision	(?)	Alive, 27 yr. 11 mo.
I-A	7*	Radical hysterectomy and palliative irradiation	Pos.	Alive, 5 yr. 10 mo.
I-B	13	Local excision	(?)	Alive, 40 yr. 4 mo.
I-B	21	Radical hysterectomy and vaginectomy (vaginal extension)	Neg.	Alive, 3 yr. 5 mo.
I	75	Exploration only	Pos.	Dead

*Associated maternal diethylstilbestrol treatment.

Table IV. Survival among 11 patients who had clinical Stage II clear-cell adenocarcinoma of the cervix (Mayo Clinic, 1910-1972)

Stage	*Age at diagnosis (yr.)*	*Lymph nodes*	*Status*
Irradiation only (200 kV.):			
II-A	63	(?)	Dead (4 yr. 7 mo.)*
II-B	32	Pos.	Dead (5 yr. 1 mo.)*
II	21	(?)	Dead (5 yr. 7 mo.)*
Radical hysterectomy:			
II-A	14	Pos.	Dead (4 yr. 6 mo.)*
II-A	15†	Neg.	Alive, 7 yr. 1 mo.
II-A	19‡	Neg.	Alive, 2 yr. 2 mo.
II-A	29	Pos.	Alive, 5 yr. 8 mo.
II-A	36	Neg.	Alive, 18 yr. 4 mo.
II-B	10	Neg.	Alive, 10 yr. 1 mo.
II-B	23	Neg.	Alive, 4 yr. 2 mo.
Radical hysterectomy and irradiation:			
II-A	19†	Pos.	Dead (2 yr. 4 mo.)*

*Time in parentheses represents survival time.

†Associated maternal diethylstilbestrol treatment.

‡Associated maternal diethylstilbestrol treatment uncertain.

Table V. Survival among nine patients with clinical Stage III clear-cell adenocarcinoma of the cervix (Mayo Clinic, 1910-1972)

Treatment	*Age at diagnosis (yr.)*	*Status*
Irradiation only (radium + 200 kV.)	46	Dead (11 yr. 6 mo.)*
Irradiation only (radium + 200 kV.)	65	Dead (5 yr. 10 mo.)*
Irradiation only (radium + 200 kV.)	35	Dead (1 yr. 8 mo.)*
Radium	82	Dead (1 yr. 10 mo.)*
^{60}Co	63	Dead (1 yr. 3 mo.)*
External radiation only (? type)	55	Dead (6 yr.)*
Radium + internal radiation‡	40	Alive (11 yr. 9 mo.)* (9 yr. 1 mo.)†
Radium + ^{60}Co‡	55	Alive (10 yr. 2 mo.)* (7 yr. 5 mo.)†
Radium + ^{60}Co‡	51	Dead (2 yr. 1 mo.)* (1 yr. 5 mo.)†

*Time in parentheses represents survival time after diagnosis.

†Survival after exenteration.

‡Irradiation followed by exenteration for recurrence.

addition to the radical hysterectomy, the patients with vaginal tumors underwent total vaginectomy and subsequent vaginal reconstruction during the postoperative period.

Comment

Although the conclusions that can be drawn from a small series such as ours are limited, the data indicate several trends that may be helpful in planning therapy. First, clear-cell adenocarcinoma confined to the vagina can be managed successfully with radical surgery. The prognosis seems to be better than that for patients with squamous-cell cancer of the same stage.[9] Second, because three patients with clinical Stage I and four with clinical Stage II clear-cell adenocarcinomas of the cervix were found at laparotomy to have lesions that

Table VI. Delay in diagnosis in 36 cases of vaginal and cervical clear-cell adenocarcinoma

No. of patients	*Stage*	*Delay (mo.)**
Vaginal adenocarcinoma:		
5	I	0,† 0,† 5, 6, 7†
1	II	0
1	IV	6
Cervical adenocarcinoma:		
5	I	0, 0, 2, 4,† 12 (mean 3.6, median 2.0)
11	II	2,† 3, 4, 4, 6,† 6, 12, 24, 24,† 60, (?) (mean 14.5, median 6.0)
9	III	0, 0, 2, 4, 6, 18, 40, (?), (?) (mean 10.0, median 4.0)
4	IV	(?), (?), (?), (?)

*0 delay means discovered on routine examination.

†Associated maternal diethylstilbestrol treatment.

Table VII. Survival among patients who had diethylstilbestrol-associated tumors

Stage	*Age at diagnosis (yr.)*	*Treatment*	*Lymph nodes*	*Status*
Vaginal adenocarcinoma:				
I	10	Radical hysterectomy and vaginectomy	Neg.	Alive, 7 yr.
I	20	Radical hysterectomy and vaginectomy	Neg.	Alive, 10 mo.
I	20	Radical hysterectomy and vaginectomy	Neg.	Alive, 4 mo.
Cervical adenocarcinoma:				
I-A*	7	Radical hysterectomy and irradiation	Pos.	Dead (5 yr. 10 mo.)†
II-A	15	Radical hysterectomy	Neg.	Alive, 7 yr. 1 mo.
II-A	19‡	Radical hysterectomy	Neg.	Alive, 2 yr. 2 mo.
II-A	19	Radical hysterectomy and irradiation	Pos.	Dead (2 yr. 4 mo.)†

*Tumor confined to cervical polyp, but with positive lymph nodes.

†Time in parentheses represents survival from diagnosis.

‡Associated maternal diethylstilbestrol treatment uncertain but suspected.

were "understaged," abdominal exploration would appear reasonable in all patients with clear-cell disease to determine more accurately the extent of the lesion; this will aid in the planning of appropriate and adequate therapy. Third, irradiation alone has been followed by late recurrence in all three patients who had Stage II cervical tumors treated by this modality. Generally, however, the patients who received irradiation were those who were seen earliest in this series, and this does not represent the results of the newer methods of delivering radiation therapy. Also, several of the patients who had surgery only have been followed less than 5 years, although four are long-term survivors. Fourth, irradiation was followed by recurrence in all nine patients with Stage III cervical tumors who were so treated. Because of this, exploratory laparotomy may be indicated, and an exenterative operation considered. Exenteration may provide prolonged or even permanent "salvage" in patients who have recurrent clear-cell disease (when the operation is still feasible). Fifth, no difference in survival was noted between patients who had clear-cell adenocarcinoma after maternal exposure to diethylstilbestrol and those who had clear-cell adenocarcinoma but were not exposed to maternal diethylstilbestrol.

Even when the "modern" techniques and dosages of radiation therapy are used, the vagina, bladder, and rectum sustain *some* permanent devascularization, fibrosis, and dysfunction. In the unfortunate young patient who has Stage I or II clear-cell disease, the treatment of choice appears to be primary radical hysterectomy, including total vaginectomy when indicated by the distribution of the malignancy. Satisfactory vaginal function can be provided by use of a split-thickness skin graft reconstruction, usually inserted 6 to 9 days after the initial operation.

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A quantitative study of the pregnancy zone protein in sera of women taking oral contraceptives

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By means of a single radial immunodiffusion the concentration of "the pregnancy zone protein" (PZ) was measured in sera from women taking oral contraceptive drugs. Women taking only 0.3 mg. of norethisterone were found not to induce measurable amounts of PZ, whereas women taking combined contraceptive drugs showed a significant rise in concentration of PZ. After six months' treatment, 1 mg. of norethisterone and 0.1 mg. of meastranol daily were found to give an average concentration of PZ amounting to 59 mg. per 100 ml., that is, approximately half of the concentration of PZ in sera from women during the last trimester of pregnancy. Different combined contraceptive pills gave rise to different concentrations of PZ.

THE PREGNANCY ZONE protein (PZ), recently purified with a molecular weight of 359,000[1, 2] is known to be found in sera of women during pregnancy.[3–8] In nonpregnant women or men PZ is generally not detectable by immunodiffusion test (less than 4 mg. per 100 ml.). This α_2-globulin, first described by Smithies,[9] has been found to have immunosuppressive properties.[10–12] During pregnancy PZ can be detected in increasing amounts from the end of the first trimester, and during the last trimester the average level is about 100 mg. per 100 ml. After delivery the concentration decreases rapidly.

Estrogen seems to be necessary for the induction of PZ.[2, 8] PZ is found not only in pregnant women but also in women taking oral contraceptive drugs[13–15] and in men treated with estrogen for prostatic cancer.[16, 17] Previous investigations[14, 15, 17] indicate that the occurrence of PZ is related to the composition of the drugs.

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The purpose of the present investigation was to measure quantitatively the variations of PZ in women treated with oral contraceptive drugs and to study the level of PZ in relation to the composition of the oral contraceptive drugs.

Material and methods

A total of 322 blood samples were drawn from 103 women prior to and during treatment with oral contraceptives. The blood samples were collected without anticoagulant. After coagulation the serum was decanted and stored at − 20° C. until use. All sera were coded and tested blindly.

Of the 103 women, 80 were specially selected according to certain criteria. They were all healthy and were not taking other drugs. They had not been pregnant and they had never been treated with oral contraceptives before the investigation. These 80 women were divided into two groups: twenty-two were treated with a combination of 1 mg. of norethisterone and 0.1 mg. of mestranol (Norinyl 1 + 100) and 58 women were treated with 0.5 mg. of norgestrel and 0.05 mg. of ethinyl estradiol (Ovral). Blood samples were obtained before treatment and then after one month, three months, and, in some women, also after six months of treatment.

In addition to these 80 women, a third group of 23 women were examined during treatment with low-dose progestin contraceptives. They had previously tried

Table I. Concentration of pregnancy zone protein during treatment with oral contraceptive drugs

Composition of drug	*Level of PZ mg. per 100 ml., (mean ± S.D.)*				*Per cent of women with increased amounts of PZ (> 10 mg. per 100 ml.) during treatment*
	Before	*After 1 month*	*After 3 months*	*After 6 months*	
Norethisterone, 0.3 mg. (Nor-Q.D.)	<4 ± 0 (23)	<4 ± 0 (21)	<4 ± 0 (21)	—	0
Norgestrel, 0.5 mg., ethinyl estradiol, 0.05 mg. (Ovral)	<4 ± 0 (58)	11 ± 17 (55)	17 ± 26 (43)	22 ± 29 (28)	59
Norethisterone, 1 mg., mestranol, 0.1 mg. (Norinyl 1 + 100)	<4 ± 0 (22)	22 ± 21 (21)	47 ± 37 (21)	59 ± 41 (19)	91

Numbers in parentheses are the numbers of patients.

combined oral contraceptives and wanted to change for various reasons. Pills containing 0.3 mg. of norethisterone (Nor-Q.D.) were used and blood samples were obtained after one and three months of treatment.

The serum concentration of PZ was measured by means of single radial immunodiffusion[18, 19] as described in a previous publication.[8] With this method the lowest concentration of PZ that could be accurately measured was 5 mg. per 100 ml.[8]

Results

Before treatment a measurable serum concentration of PZ (> 4 mg. per 100 ml.) was found in only six of 103 women, and there was no value exceeding 10 mg. per 100 ml.

Of the 80 women taking combined oral contraceptives, 54 were found to respond with elevated levels of PZ (> 10 mg. per 100 ml.) in the serum. The remaining 26 women on combined pills and the group of 23 women treated with progestin pills only showed no increase in the PZ concentration during the period of treatment.

Between the two groups of women taking combined pills there were marked differences. The frequency of individuals with increased amounts of PZ, that is, more than 10 mg. per 100 ml. was 91 per cent for women taking norethisterone, 1 mg., and mestranol 0.1 mg., while the corresponding figure for those taking norgestrel, 0.5 mg., and ethinyl estradiol, 0.05 mg. was 59 per cent (Table I). This difference was statistically significant ($p < 0.01$).

Furthermore, the mean values for the concentration of PZ as seen in Fig. 1 were higher in women taking Norinyl 1 + 100 than in women taking Ovral. The significance of difference after one, three, and six months was $p < 0.05$, $p < 0.05$, and $p < 0.05$, respectively. Also, when women with no detectable increase of PZ during treatment were excluded the mean value for the concentration of PZ after six months, was higher for women taking Norinyl 1 + 100, that is, 57 compared to 33 mg. per 100 ml. ($P < 0.05$).

The duration of treatment seemed to be of importance (Fig. 1 and Table I). Thus, the PZ concentration increased rapidly during the first month of treatment and thereafter the mean values were more stable and only slightly increasing. In both groups the increase of PZ was most pronounced during the first month of treatment (Norinyl 1 + 100, $p < 0.01$; and Ovral, $p < 0.01$). The further increase between one and three months was not statistically significant for Ovral, but that for Norinyl 1 + 100 was significant ($p < 0.05$). The increase between three and six months was not statistically significant for any group.

Comment

The present investigation confirms that induction of PZ is not exclusive to pregnancy but is found also during hormonal treatment, that is, combined contraceptive drugs. The mean concentration of PZ during this treatment was lower than that during pregnancy. In a previous investigation[8] the mean concentration during the last trimester of pregnancy was found to be about 100 mg. per 100 ml. The highest mean value now observed in this study (six months' treatment with Norinyl 1 + 100) was 59 mg. per 100 ml. (Table I)—approximately half the mean concentration during pregnancy. The inducing factor is most likely estrogen which is present in much higher concentrations during pregnancy than during treatment with modern contraceptive drugs. This explanation is supported by the present observation that none of the 23 women taking progestin only-contraceptives (norethisterone, 0.3 mg.) showed increased amounts of PZ. PZ is also found in men treated with estrogen.[16, 17]

There is an individual variation in the ability to produce increased amounts of PZ. In this investigation, as in previous studies, some individuals did not re-

act.[7, 8, 14] High mean values of PZ can be the result of both a high induction frequency (individual ability to react with PZ synthesis after exposure to a certain amount of estrogen) or a high induction level (the concentration of PZ may vary individually). The results in Table I show that both the induction frequency and the induction level are significantly affected by the type and dosage of the drug. The differences between the two groups of women taking Norinyl 1 + 100 and Ovral, respectively, are most likely due to the different composition of the drugs. It seems probable that the "total estrogen effect" is of fundamental importance for the induction of PZ. The lower concentrations found in women taking Ovral could be due to a straight estrogen dose response or interactions between estrogen and progestin components. Norgestrel has been reported to have an antiestrogen effect.[20]

The increased synthesis of PZ was pronounced during the first month of treatment. After the third month the increase in PZ concentration was still measurable but rather slow (Fig. 1). This is in agreement with the previous observation that no increase in the occurrence of PZ was found after six months of treatment with oral contraceptive drugs.[14]

The biological role of PZ is partly unknown but this protein is known to have immunosuppressive properties in physiologic concentrations, that is, PZ can inhibit the lymphocyte response to phytohemagglutinin[10] and also the mixed lymphocyte reaction.[11, 12] In the present investigation, PZ was found in increased amounts during treatment with combined oral contraceptives. The immunosuppressive properties of this protein may be related to the recently presented data that some viral diseases are more common in women taking oral contraceptives.[21] Furthermore, plasma from women treated with oral contraceptives has been reported to suppress the mixed lymphocyte reaction.[22]

It is possible that oral contraceptives may have an immunosuppressive effect in some but not all women.

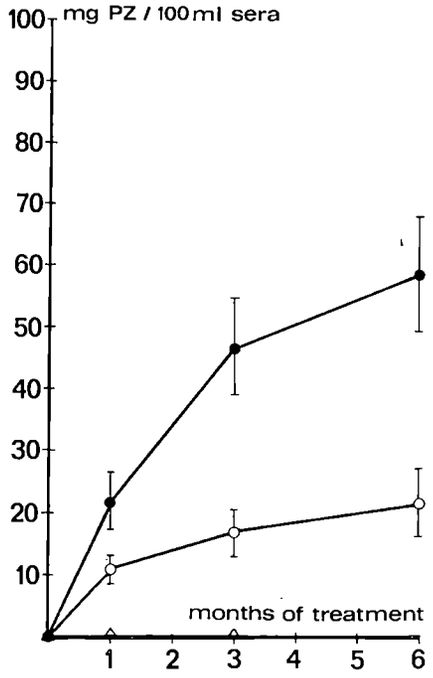


Fig. 1. Concentration of pregnancy zone protein (mean ± S.E.M.) in sera of women during treatment with oral contraceptive drugs. Δ, Norethisterone, 0.3 mg. (Nor-Q.D), ○, Norgestrel, 0.5 mg. ethinyl estradiol, 0.5 mg. (Ovral). ●, Norethisteron 1 mg., mestranol, 0.1 mg. (Norinyl 1 + 100).

These new aspects make it imperative to study in more detail the individual variations in PZ induction and also possible genetic factors controling variations in the individual response.

The increase of PZ during pregnancy both in women and in some animals[11, 23] certainly is a physiologic phenomenon. The increase of the same protein during hormonal treatment may be regarded as a side effect of unknown implications.

Our thanks are due to Professors L. Beckman and P. Lundström for stimulating criticism and advice.

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The age of occurrence of gonadal tumors in intersex patients with a Y chromosome

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A total of 320 intersex patients with a Y chromosome were classified into four groups; (1) gonadal dysgenesis, (2) asymmetrical gonadal differentiation, (3) virilizing male hermaphroditism, and (4) feminizing male hermaphroditism (testicular feminization syndrome). Of these 320 cases, 98 were from the files of The Johns Hopkins Hospital and the remainder from the literature. The incidence of tumors in relation to age and clinical classification was analyzed by computer. The results were plotted for each group. It was found that the percentage of tumors rose appreciably soon after the age of puberty in the first three groups, and it was concluded that the gonads were best removed before the age of puberty. In the case of testicular feminization patients, procrastination until the age of 25 could be considered, if one were willing to assume the risk of neoplasia of about 3.6 per cent until then.

AN INTERSEX patient with a Y chromosome can be encountered in at least four clinical situations: (1) gonadal dysgenesis, (2) asymmetrical gonadal differentiation, (3) virilizing male hermaphroditism, and (4) testicular feminization.

From the beginning of this century, it has been appreciated that gonadal tumors are more prone to occur in dysgenetic gonads.[1–7]

In recent times, the malignant potential of the dysgenetic gonads in the intersex patient with a Y chromosome has been stressed by many authors.[8–18]

The commonest tumors encountered in dysgenetic gonads have been the dysgerminoma and the gonadoblastoma. While the dysgerminoma can arise in women with normal ovaries, the gonadoblastoma almost always arises in women with dysgenetic gonads. The dysgerminoma, a term coined by Meyer,[19] is a tumor which most observers believe arises from the germ cells in the female and is thus analagous to the seminoma in the male.

The gonadoblastoma was first described by Scully[20] in 1953. It is a gonadal tumor composed of germ cells, sex cord derivatives resembling granulosa or Sertoli cells, and sometimes stromal elements resembling lutein or Leydig cells. This name was chosen by Scully because the neoplasm appeared to recapitulate gonadal development more completely than any other type of tumor. Scully[21] in 1970 reviewed 74 cases of gonadoblastomas and found that a Y chromosome was present in all but three of the cases where chromosomal analyses were available.

To some extent the frequency of neoplasia may be exaggerated since certain cases of gonadal dysgenesis with a Y chromosome might never be recognized except for the clinical appearance of a pelvic tumor and a resulting laparotomy. On the other hand, bilateral germ cell tumors may completely replace the gonadal streaks and the diagnosis of gonadal dysgenesis may be missed.[22]

Despite the documentation of a large number of females with a Y chromosome, dysgenetic gonads, and gonadal tumors, the data have been derived from several series with only a few cases. Therefore, it has not been possible to state the risk according to age. The present study, using cases from the literature and from

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Table I. Group I(a): Gonadal dysgenesis with a Y chromosome (unbiased); type of tumor

Type of tumor	*No. of cases*	*Reference*
Gonadoblastoma	7	This series, 18, 25, 26, 27, 28, 65
Dysgerminoma	3	18, 23, 24
Gonadoblastoma and dysgerminoma	1	18
Gonadoblastoma and dysgerminoma with embryonal carcinoma and choriocarcinomatous elements	1	This series
Total	12	

Table II. Group I(a): Gonadal dysgenesis with a Y chromosome (unbiased); chromosomal complement

Karotype	*No. of cases*	*Reference*
46,XY	43	This series, 17, 18, 22, 23, 24, 26, 27, 28, 29, 30, 31, 32, 34, 35, 36,
46,X,Yq-	1	37
46,XY,dic(Y)	2	25, 65
45,X/46,XY	21	This series, 18, 24, 25, 29, 38, 39, 40
46,XX/46,XY	1	24
45,X/47,XYY	1	40
45,X/46,XY/47,XYY	1	24
45,X/46,X,dic(Y)	1	65
47,XY,+G	1	33
45,X,-16,+t(16;Yp-)/46,X,Yq-,-16,+t(16;Yp-)	1	This series
Total	73	

Table III. Group I(b): Gonadal dysgenesis with a Y chromosome (biased); type of tumor

Type of tumor	*No. of cases*	*Reference*
Gonadoblastoma	22	13, 14, 41, 45, 46, 57, 51, 52, 53, 54, 55, 56, 58, 59, 60, 61, 65
Dysgerminoma	11	13, 14, 41, 42, 43, 44, 35
Gonadoblastoma and dysgerminoma	3	62, 63
Gonadoblastoma and/or dysgerminoma with choriocarcinomatous elements	2	13, 14
Gonadoblastoma and dysgerminoma with embryonal carcinoma	1	49
Unknown	1	61
Brenner	1	13
Total	41	

the files of The Johns Hopkins Hospital, was carried out to determine the expectancy of neoplasia in the gonads of these intersex patients with Y chromosome in relation to age. This is important in order to be able to state with some confidence the age by which dysgenetic gonads should be removed to avoid serious risk from tumor.

Materials and methods

A total of 320 intersex patients with a Y chromosome were classified into the following four groups: (1) gonadal dysgenesis, (2) asymmetrical gonadal differentiation, (3) virilizing male hermaphroditism, and (4) testicular feminization (complete type).

Many cases were available from the files of The Johns Hopkins Hospital and, whenever possible, pertinent series of cases from the literature were included, giving consideration to the following two criteria: (1) the age of the patient at laparotomy or when seen was available, and (2) chromosome studies were known.

For some categories, an unbiased and a biased series were derived. The cases were considered to be relatively unbiased for our purposes if they were ascertained on the basis of their gonadal or chromosomal abnormality. On the other hand, they were considered biased if the ascertainment was on the basis of the discovery of a tumor.

Group I(a): Gonadal dysgenesis, unbiased series. In the files of The Johns Hopkins Hospital there were 11 cases, of which two had tumors. One was in a 15-year-old girl with bilateral streaks which had gonado-

Table IV. Group I(b): Gonadal dysgenesis with a Y chromosome (biased); chromosomal complement

Karyotype	*No. of cases*	*Reference*
46,XY	30	13, 14, 41, 42, 44, 45, 47, 51, 52, 53, 55, 56, 62, 63, 85
46,X,dic(Y)		65
45,X/46,XY	5	13, 46, 58, 59
46,XX/46,XY	1	43
45,X/47,XYY	2	54, 60
45,X/46,XY/47,XYY	2	61
45,XY,-D,-G,+t(DqGq)	1	49
Total	41	

Table V. Group II(a): Asymmetrical gonadal differentiation (unbiased); type of tumor

Type of tumor	*No. of cases*	*Reference*
Gonadoblastoma	2	66, 93
Total	2	

blastomas. Her karyotype was 45,X,-16, + t(16; Yp-)/46,X,Yq-,-16, + t(16;Yp-). The second patient was 16 years old with 46,XY chromosomes. The left streak had no tumor. On the right side was a gonadoblastoma with overgrowth of malignant germ cells, principally embryonal carcinoma and a focus of dysgerminoma. There was metastatic embryonal carcinoma in the omentum. She had a recurrence 12 months later in the cul-de-sac and the left lumbar gutter. This was found to be dysgerminoma with tubular Sertoli cell pattern and a focus of trophoblast. Her condition worsened in spite of chemotherapy.

Included were 62 pertinent cases from the literature where ascertainment seemed to be on the basis of gonadal dysgenesis rather than on tumors. There were 12 cases of tumors among these. Table I shows the type and distribution of these tumors. Table II shows the chromosomal complement of these 73 cases. The youngest patient without a tumor was 5.5 years old and the oldest was 56 (Fig. 1). The youngest patient with a tumor was 5 years old and the oldest was 24.

Group I(b): Gonadal dysgenesis, biased series. There were 41 tumor reports in this subseries where ascertainment was based on tumor rather than on dysgenesis (Fig. 2). Table III shows the type and distribution of tumors. Table IV shows the chromosomal complement of these 41 cases. The youngest patient with a tumor was 6 years old and the oldest was 43 (Fig. 1).

Group II(a): Asymmetrical gonadal differentiation, unbiased series. There were two cases in the files of The Johns Hopkins Hospital and neither had a tumor.

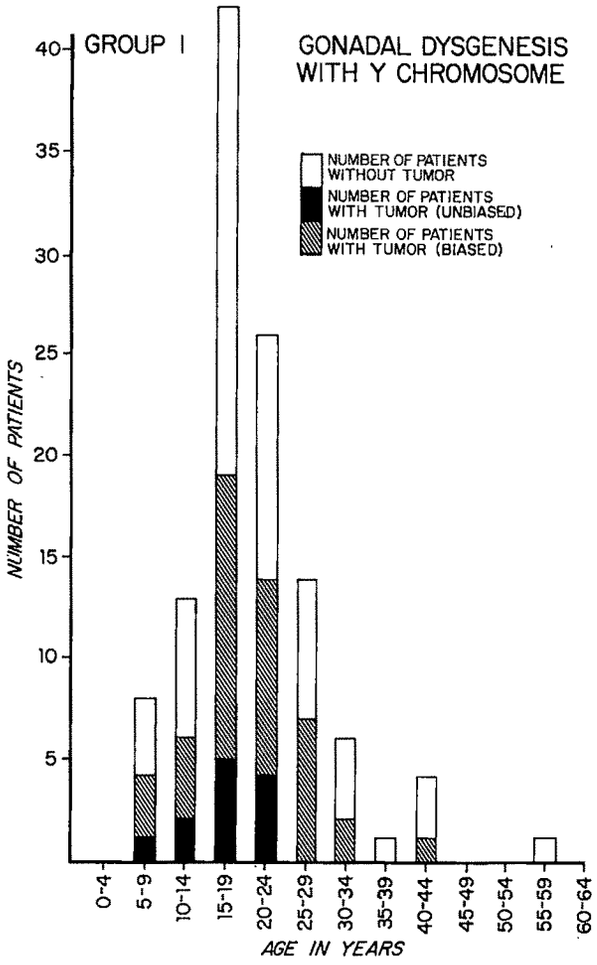


Fig. 1. The age distribution of patients without a tumor, with a tumor (unbiased), and with a tumor (biased) in Group I.

The karyotype of one was 46,XY and the other 45,X/46,X,Yq-. There were 49 pertinent cases from the literature, making a total of 51 cases in all; of these, two had tumors. Table V shows the type and distribution of these tumors. Table VI shows the chromosomal complement of these cases.The youngest patient in the series without a tumor was a newborn infant and the oldest was 47 years old. The youngest patient with a tumor was 19 years old, and the oldest was 21 (Fig. 2).

Group II(b): Asymmetrical gonadal differentiation, biased series. There were eight cases of tumors reported in the literature. Table VII shows the type and distribution of tumors. Table VIII shows the chromosomal complement of these eight cases. The youngest patient with a tumor was 9 years old, and the oldest was 26 (Fig. 2).

Group III: Virilizing male hermaphroditism. There were 62 cases in the files of The Johns Hopkins Hospital, of which two had tumors. Table IX shows the

Table VI. Group II(a): Asymmetrical gonadal differentiation (unbiased); chromosomal complement

Karyotype	*No. of cases*	*Reference*
46,XY	19	This series, 64, 67, 69, 70, 71, 87
45,X	2*	86
45,X/46,XY	18	13, 18, 29, 37, 38, 64, 74, 75, 86
45,X/46,X,Yq-	1	This series
45,X/47,XYY	1	18
45,X/48,XXXY	1	78
45,X/46,X,Yq-	1	77
45,X/46,X,Yq+	1	66
45,X/46,X,dic(Y)	2	65
45,X/46,XX/46,XY	1	73, 86
45,X/46,XY/46,X,dic(Y)	2	66
45,X/46,X,i(Yq)/47,X,i(Yq),Yq-	1	79
45,X/45,X,-D,+t(DqYq)	1	68
Total	51	

*These were included here because of the presence of the testis on one side.

Table VII. Group II(b): Asymmetrical gonadal differentiation (biased); type of tumors

Type of tumor	*No. of cases*	*Reference*
Gonadoblastoma	6	57, 69, 76 82, 83, 84
Seminoma	1	80
Gonadoblastoma and dysgerminoma with embryonal carcinoma	1	72
Total	8	

type of these two tumors. The chromosomal complement of all of these patients was 46,XY. The youngest patient in the series without a tumor was 1 year old and the oldest was 46. The youngest patient with a tumor was 15 years old and the oldest was 38 (Fig. 3).

Group IV: Testicular feminization syndrome (complete type). There were 23 cases in our files and none of them had a tumor. There were 82 cases available from the literature, making a total of 105 cases. There were seven malignant tumors among them. Table X shows the type and distribution of these seven tumors.

The karyotype of these patients was usually 46,XY. The youngest patient without a tumor was 4 months old and the oldest was 70 years old. The youngest patient with a tumor was 14 years old and the oldest was 51 (Fig. 4).

Results

The data were computerized[48, 50] and the results were plotted with age on the abscissa and the expectancy of tumors on the ordinate. The sigmoid curves were applied to data points (Figs. 5 and 6).

Table VIII. Group II(b): Asymmetrical gonadal differentiation (biased); chromosomal complement

Karyotype	*No. of cases*	*Reference*
45,XY	4	69, 72, 76, 82
45,X/46,XY	2	57,83
45,X/46,X,Yq-	1	84
45,X/46,XX/46,XY	1	80
Total	8	

Table IX. Group III: Virilizing male hermaphroditism; type of tumor

Type of tumor	*No. of cases*	*Reference*
Gonadoblastoma	1	This series
Seminoma	1	This series
Total	2	

Table X. Group IV: Testicular feminization syndrome; types of tumor

Type of tumor	*No. of cases*	*Reference*
Seminoma	2	3
Dysgerminoma	1	3
Teratoma	1	3
Arrhenoblastoma	1	3
"Alveolar cancer"	1	3
"Pelvic cancer"	1	3
Total	7	

Gonadal dysgenesis. In the unbiased series designated as SSY in Fig. 5 the expectancy of tumors is 2 per cent at the age of 10 years, 5 per cent at the age of 14, 16 per cent at the age of 20, and 27.5 per cent at the age of 30. If we include the biased series designated as SSY(b) the resultant curve in Fig. 5 rises more steeply than the unbiased curve SSY. The expectancy of tumors is four per cent at the age of 10 years, 10 per cent at the age of 15, 73 per cent at the age of 30, and 80 per cent at the age of 40 (Fig. 5).

Asymmetrical gonadal differentiation. In the unbiased series designated as STY in Fig. 5 the expectancy of tumors is 10 per cent at the age of 20 years and 19 per cent at the age of 30 years. In the biased series, designated as STY(b) the expectancy of tumors is 3 per cent at the age of 10 years, 10 per cent at the age of 13, 20 per cent at the age of 15, and 74.5 per cent at the age of 26 (Fig. 5).

Virilizing male hermaphroditism. The expectancy of tumors is 3 per cent at the age of 20 years but then rises sharply to 46 per cent at the age of 40 years (Fig. 6).

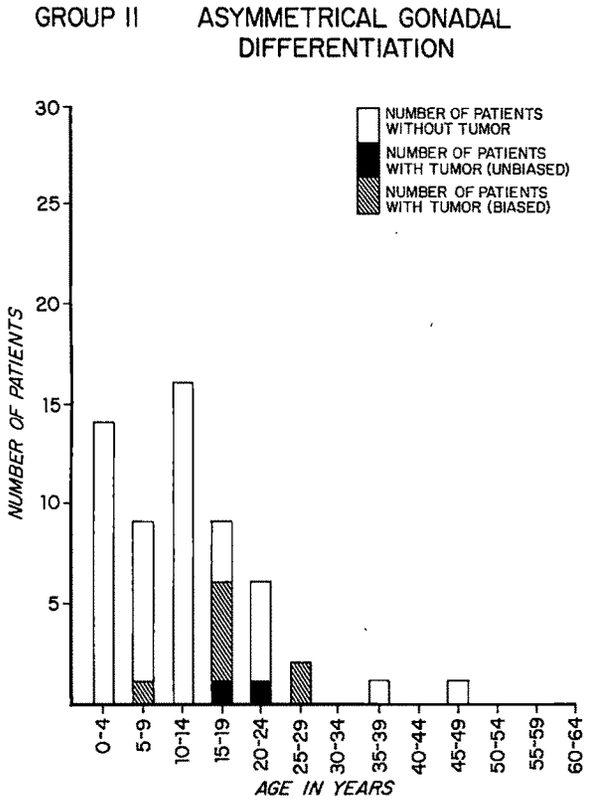


Fig. 2. The age distribution of patients without a tumor, with a tumor (unbiased), and with a tumor (biased) in Group II.

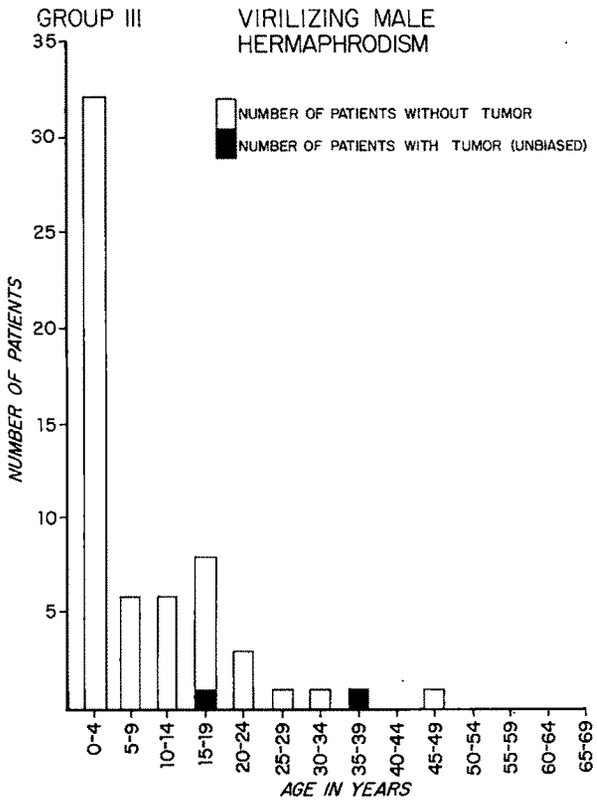


Fig. 3. The age distribution of patients without a tumor and with a tumor (unbiased) in Group III.

Testicular feminization (complete form). The expectancy of tumors is 3.6 per cent at the age of 25 years and this reaches 33 per cent at the age of 50. The curve here is shifted to the right as compared to the other categories of patients (Fig. 6).

Comment

It seems clear from the curves, obtained in Figs. 5 and 6 above, that the expectancy of tumors rises appreciably at about puberty in three groups of intersex patients with a Y chromosome: gonadal dysgenesis, asymmetrical gonadal differentiation, and male hermaphroditism. Hence, it may be concluded that the gonads are best removed from these patients by the age of puberty. Unfortunately, data are not available to further break down these groups, particularly male hermaphroditism, which is a heterogeneous group, so that for the moment nothing can be said about the various subgroups in these major groups with respect to tumor formation. In testicular feminization patients, on the other hand, if a risk of something less than 4 per cent is acceptable, delay in removal of the gonads may be entertained until approximately the age of 30. This has some advantages in allowing this type of patient to spontaneously feminize. This conclu-

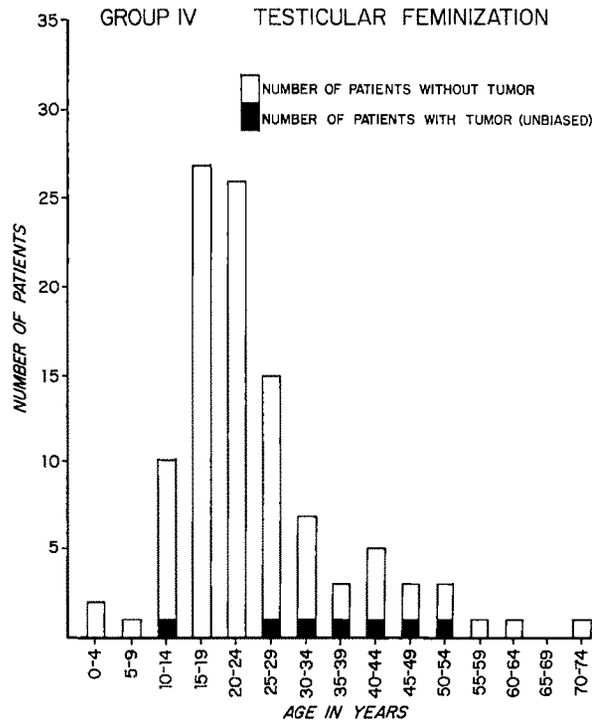


Fig. 4. The age distribution of patients without a tumor and with a tumor (unbiased) in Group IV.

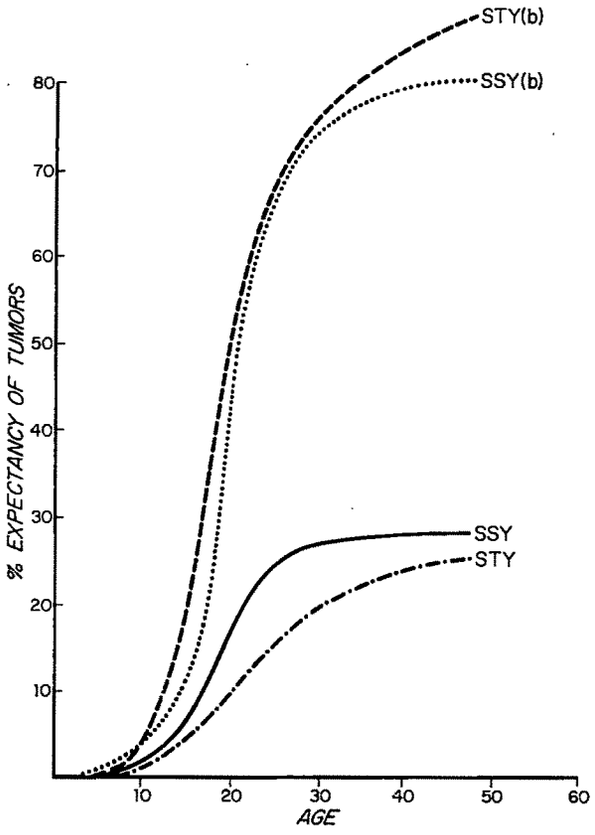


Fig. 5. The percentage expectancy of tumors in relation to age in gonadal dysgenesis and asymmetrical gonadal differentiation. *SSY* denotes bilateral streaks with Y chromosome; *STY* denotes streak and testis with Y chromosome; *(b)* denotes biased group.

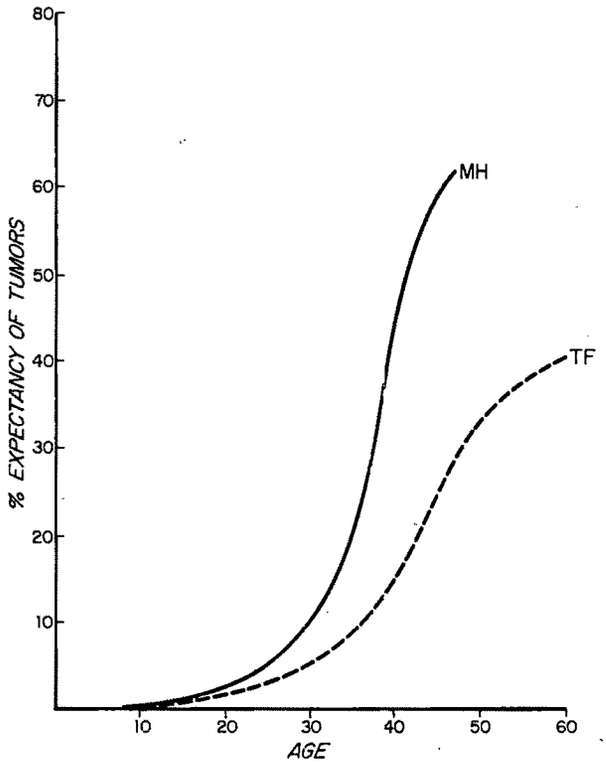


Fig. 6. The percentage expectancy of tumors in relation to age in virilizing male hermaphroditism *(MH)* and testicular feminization *(TF)*.

sion is in line with that reached by Morris and Mahesh[10] who, in their review, found one malignant tumor in a teenage patient, two in those in their twenties, and 11 in 50 reported cases of 30 years or older. They thought that this 22 per cent incidence of malignancy was probably high, because the presence of the tumor was the indication for operation in many instances.

Dewhurst,[81] on the other hand, found no malignancies in 82 patients with this syndrome. However, 41 had had bilateral orchidectomy: six before puberty, 10 between the ages of 16 and 19, 16 between 20 and 29, and nine over the age of 30. From his personal experience and from a review of the literature, he suspected that the risk of tumor was approximately 5 per cent.

The data on which the discussion of the preceding paragraph was based were collected from patients who had a karyotype of 46,XY, or some minor variation thereof. The incidence of tumor in patients with the much more common 45,X karyotype associated with intersexuality is extremely rare.

Only a very few cases of intersexuality have been reported with a tumor and a karyotype without a definite Y chromosome. These cases have been with 46,XX karyotype,[88, 89, 91] 45,X,[29, 90] 45,X/46,X,?Xq-, 45,X/46,X, + m (m being a minute metacentric chromosome),[8] and 45,X/46,XX.[92] It is always possible that an XY cell line could have been missed in some of these patients, or that a fragment from a Y chromosome could have been translocated and not discovered by techniques in vogue at the time the case was reported.

This restudy of the literature stresses again the importance of removing the gonads of intersex patients with the Y chromosome because of the strong association of the genesis of tumor in the dysgenetic gonads with the presence of a Y chromosome. The number of gonadal tumors in intersex patients, without a Y chromosome, is so small that the possibility must be entertained that in these tumor cases, a restudy with the use of contemporary techniques would reveal the presence of a Y or a translocated fragment thereof.

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Cyclic adenosine monophosphate–induced changes in the surface morphology of diapausing blastocysts and the effects on implantation

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Estrogen induces blastocyst implantation in diapausing female mice, increases uterine levels of adenyl cyclase and cyclic adenosine monophosphate (cyclic AMP), and stimulates synthesis of ribonucleic acid and protein in both the uterus and blastocyst. Furthermore, the surface ultrastructure of trophoblast cells seen with scanning electron microscopy (SEM) changes with activation of the diapausing blastocyst by estrogen. Cyclic AMP-activated blastocysts were investigated with the use of SEM and compared to blastocysts activated by estrogen. Intraperitoneal administration of cyclic AMP was generally ineffective, whereas administration of cyclic AMP intraluminally in the uterus effectively mimicked the early estrogen activation. These findings are discussed in relation to other known activation changes in preimplantation blastocysts and with regard to similar findings after administration of various antiestrogens.

THE ROLE OF estrogen in implantation of mouse and rat blastocysts is undisputed. This is evident in a recent review paper by McLaren[1] concerning activation of the blastocyst. For example, in diapausing female mice a single stimulus of estrogen induces trophoblast transformational changes,[2] activates tyrosine amino transferase in trophoblast cells while altering their surface coat properties,[3] and increases de novo ribonucleic acid (RNA) and protein synthesis in all blastocyst cells.[4–6] Further, the rapid estrogen activation of blastocysts is clearly demonstrated by the changes in trophoblast cell surface structure as seen in scanning and transmission electron microscopy.[7–9]

The relationship between estrogen, cyclic AMP, and adenyl cyclase in reproduction also appears firmly established. In 1967, Szego and Davis[10] reported that estrogen administration to rats acutely increases the uterine levels of cyclic AMP, and later Rosenfeld and O'Malley[11] demonstrated that estrogen stimulates uterine adenyl cyclase activity.

Since it appears that estrogen functions initially through the adenyl cyclase–cyclic AMP system, it should be possible to mimic estrogenic effects to some extent by cyclic AMP. Considerable work now shows that de novo RNA[5, 12, 13] and protein synthesis can be stimulated in the rat uterus by both estrogen and cyclic AMP.[14–17]

The present experiments were designed to determine whether the estrogen-induced trophoblast cell surface changes seen in the scanning electron microscope (SEM) could be reproduced with cyclic AMP and to determine if cyclic AMP is able to induce blastocyst implantation.

Material and methods

NMRI strain (Bethesda) albino mice were maintained under a controlled ten-hour night centered on midnight and fed a standard diet ad libitum. Virgin females weighing 20 to 25 grams were mated with fertile males and bilaterally overiectomized under sodium pentobarbital anesthesia on Day 3 of gestation, a vaginal plug having been found on Day 1. At

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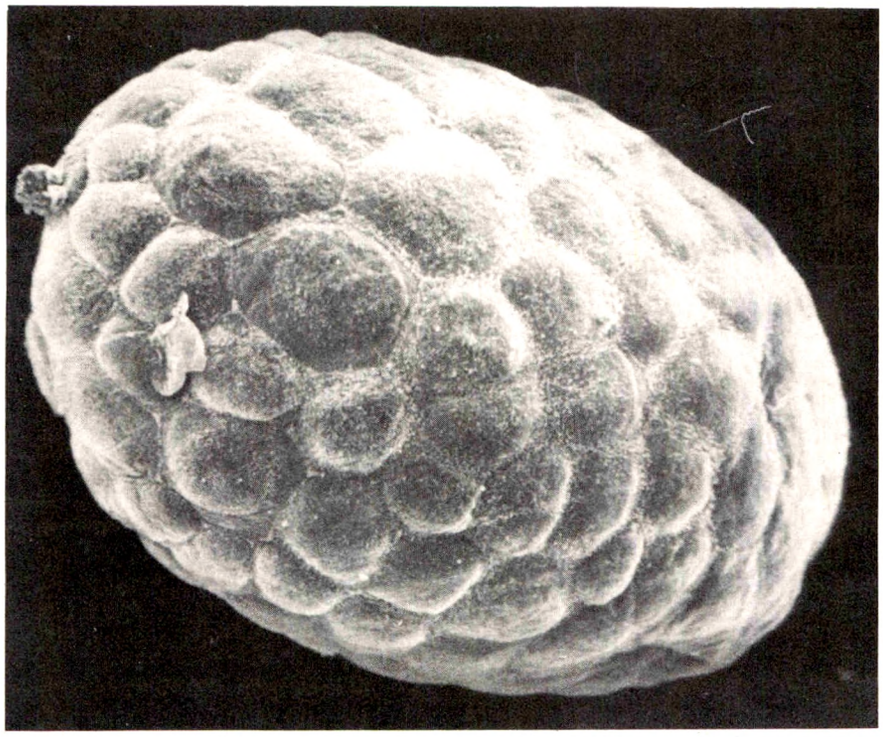


Fig. 1. Blastocyst in delay of implantation after intraluminal instillation of cyclic AMP. Signs of activation are obvious with bulging trophoblast cells, few remaining imprints, and distinct polarity, the abembryonic pole being to the left. ×750.

Table I. Single dose of cyclic AMP on Day 8

Cyclic AMP dosage	*Autopsy day*	*No. of mice*		*No. of blastocysts collected*
		Experimental	*Control*	
8M mole solution, 10 μl/uterine horn (intraluminal)	9	10	5	18 23
10 mg. in 0.5 ml./mouse (intraperitoneal)	9	5	3	24 13
5 mg. in 0.25 ml./mouse (intraperitoneal)	9	8	2	35 10

ovariectomy, 0.5 mg. of long-acting progesterone (Depo-Provera, The Upjohn Co., 50 mg. per milliliter) was administered subcutaneously producing experimental diapause.[18]

The first series of mice were treated with dibutyryl adenosine 3^1, 5^1-cyclic monophosphate (Sigma, Lot no. 42C-7540) either intraluminally in the uterus or intraperitoneally for 24 hours (Table I). In the former group the cyclic AMP or the control vehicle, sterile physiologic saline, was instilled by microsyringe into the uterine lumen on Day 8 at laparotomy after the cervix had been ligated to prevent leakage. The latter groups received intraperitoneal cyclic AMP in a single dose on Day 8 according to Table I. Effective doses of cyclic AMP were previously determined by Holmes and Bergström.[19]

At autopsy on Day 9, 24 hours later, the uteri were carefully excised, examined for implantation sites, and then flushed with 2.5 per cent glutaraldehyde in 0.1M Soerensen's phosphate buffer at pH 7.4. The blastocysts collected were rinsed in phosphate buffer, postfixed for at least 30 minutes in buffered one per cent OsO_4, rinsed in three baths of redistilled water, and prepared by freeze drying, as described previous-

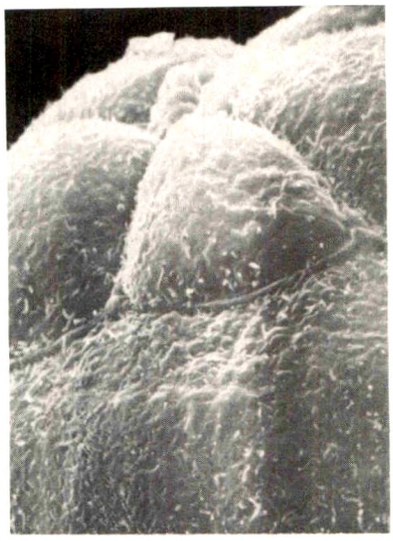


Fig. 2. Abembryonic pole of a blastocyst in delay of implantation after intraluminal instillation of cyclic AMP. Compare Figs. 2 and 3. Note absence of imprints. ×1,700.

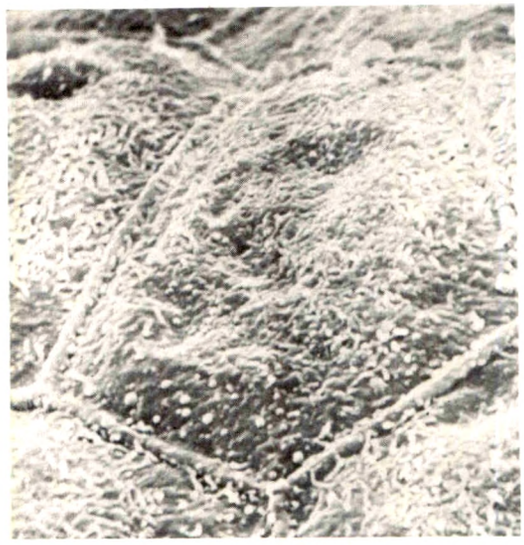


Fig. 3. Blastocyst in delay of implantation activated by 0.1 μg estradiol-17β and recovered 16 hours after estrogen was given. Abembryonic pole. Note distinct cell borders and vanishing shallow imprints on bulging trophoblast cells. ×3,000. (From Bergström, S., and Nilsson, O.: J. Reprod. Fertil. 44: 117, 1975, with due permission from the publisher.)

ly,[20] for observations in a Jeol JSM-U3 scanning electron microscope at 15 kv.

A second series of mice received 2.5 mg. of cyclic AMP in 0.25 ml. of sterile physiologic saline at pH 7.4 intraperitoneally every six hours for either 24 or 48 hours (Table II). The controls were administered 0.25 ml. of the saline vehicle only under identical conditions. The mice then were anesthetized and infused with 0.1 ml. of one per cent pontamine blue solution via the tail vein; five minutes later the uteri were observed for implantation sites. The uteri were excised carefully and flushed with glutaraldehyde to collect the blastocysts. These blastocysts also were processed as described above for scanning electron microscopy.

All observations made with SEM were done with the use of a double-blind method, and the blastocysts were judged according to characteristics previously described by Bergström.[7, 8]

Results

The results are summarized in Tables I and II, and all blastocysts observed by SEM were scored according to surface ultrastructure. The pontamine blue reaction seen early during normal implantation was not observed in any cyclic AMP–treated or control mice in this work.

The essential surface changes induced by cyclic AMP activation of blastocysts in delay of implantation (Figs. 1 and 2) were found to be similar to those seen after estrogen induction (Fig. 3). Examples of the unstimulated control blastocysts can be seen in Figs. 4 and 5. Therefore, the observed changes due to cyclic AMP activation were classified according to the features described for blastocysts activated by estrogen.[7, 8] Three categories were used: activated (bulging trophoblast cells, distinct intercellular borders, relative increase in microvilli, absence of imprints); inactive (signs of intimate proximity of the uterine epithelium to the blastocyst such as imprints, ridges, more or less masked intercellular trophoblast borders, and few microvilli); and, finally, an intermediate category of blastocysts falling between the two described.

Intraperitoneal administration of cyclic AMP was generally ineffective in producing an estrogen-like activation of the blastocyst. Even a dose of 10 mg. induced no visible surface changes in the blastocyst. A slight effect was obtained by dividing the 10 mg. into four doses of 2.5 mg. given every six hours. No additional effect was seen when the schedule was 2.5 mg. every six hours for 48 hours. Furthermore, there is always an insignificant fraction of spontaneously activated blastocysts seen in the control groups.

Intraluminal administration of cyclic AMP was clearly more effective in mimicking the activation changes induced by estrogen (Figs. 1 and 2). An evident change took place about 30 hours after an intraluminal dose of 0.025 mg., while little or no effect was seen after administration of the corresponding control vehicle.

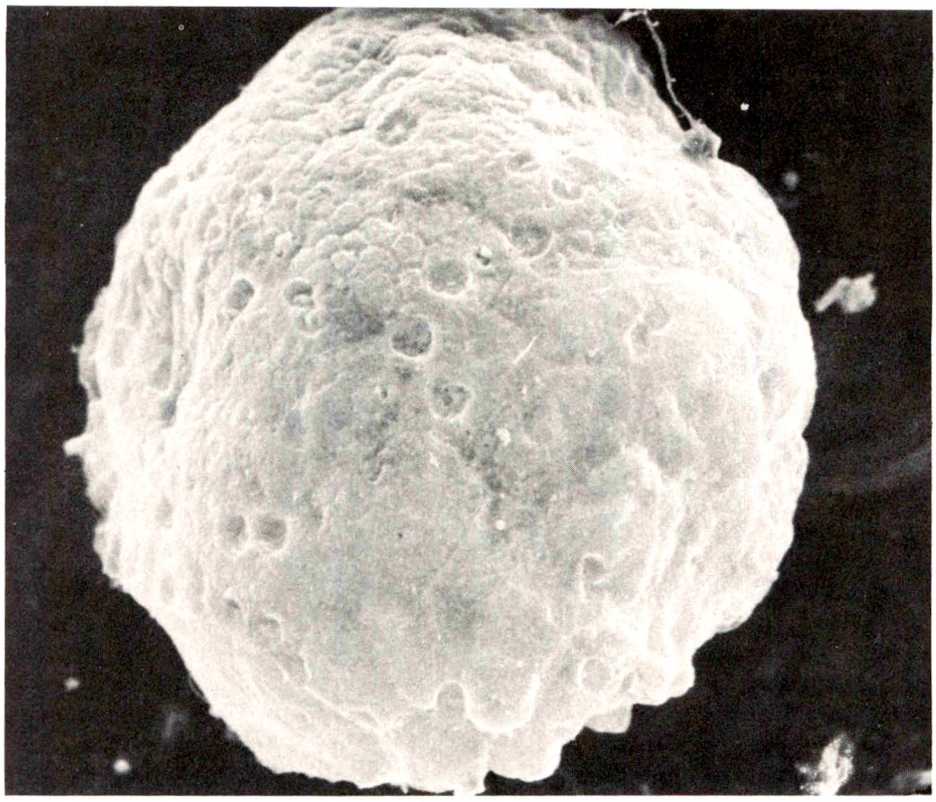


Fig. 4. Blastocyst in delay of implantation without any signs of activation. Note rough surface and multiple imprints. Some intercellular borders between trophoblast cells are barely visible. ×700.

Table II. Multiple doses of cyclic AMP from Day 8

Duration of intraperitoneal treatments (hr.) of 2.5 mg. of cyclic AMP in 0.25 ml. saline every 6 hours	*Autopsy day*	*No. of mice*		*No. of blastocysts collected*
		Experimental	*Control*	
24	9	31	14	103 61
48	10	23	11	102 45

Comment

A direct effect of estrogen on the blastocyst just before and during the implantation process as yet has not been demonstrated. We do know that estrogen stimulates uterine adenyl cyclase activity,[11] increases the uterine levels of cyclic AMP,[10] and therefore apparently functions in the uterus through the first and second messenger system referred to by Robison and Sutherland.[21] In addition, we know that estrogen, directly or indirectly, induces the blastocyst to implant[22] and, when doing so, induces numerous intra- and extracellular trophoblast changes.[3, 7] The present results demonstrate that some of these estrogen-induced changes can also be induced by cyclic AMP. Although it seems unlikely that the first and second messenger systems are not functional in blastocysts, it still cannot be shown that estrogen is the first messenger for blastocysts. However, the evidence is accumulating and other relationships would be clarified if a direct estrogen effect on blastocysts could be demonstrated and if the adenyl cyclase–cyclic AMP system was involved in the trophoblast cells.

Increased cell-to-cell attraction and adhesion can be induced under the stimulation of steroid hormones.[23] Thompson, Tomkins, and Curran[24] induced increased tyrosine aminotransferase (TAT) activity in cultured cells also with the use of steroid hormones. These two findings were linked by Ballard and Tomkins[25] who

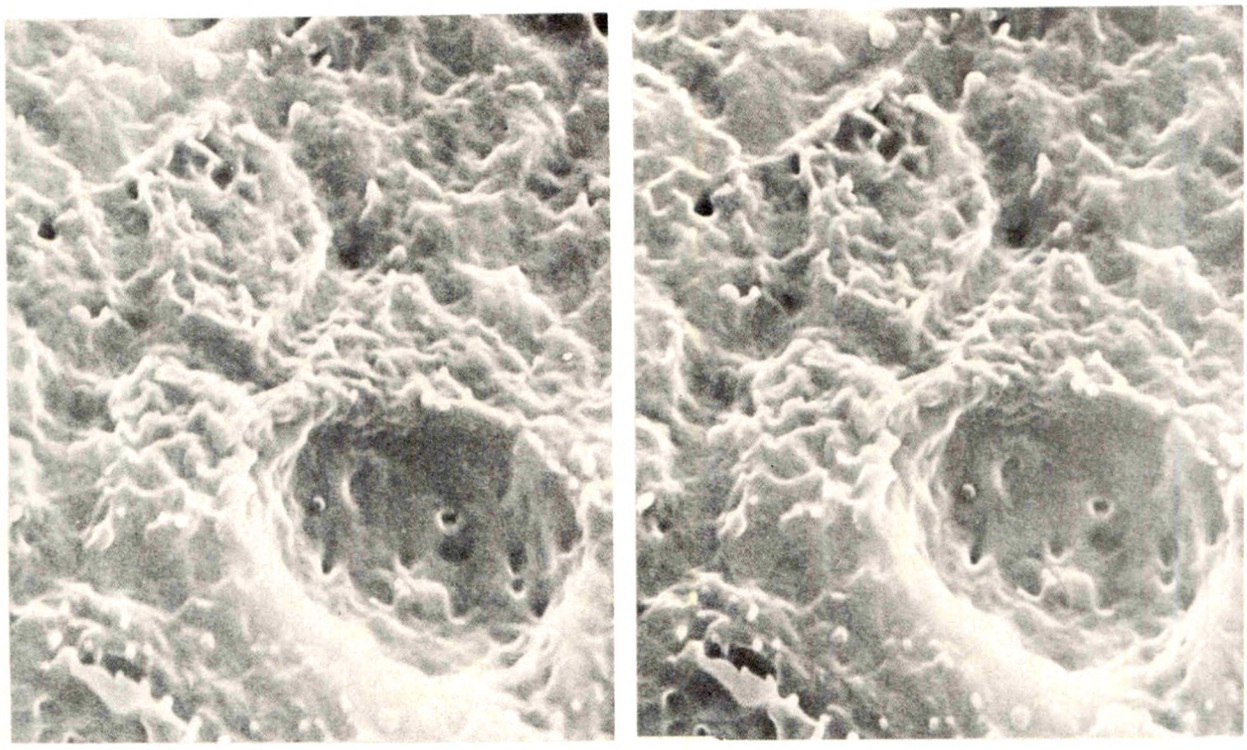


Fig. 5. Imprints on the specimen showed in Fig. 1. Stereo recording with six-degree tilt difference between the left and right figures. Note shallow imprint (upper left) due to uterine epithelial cell apices (polygonal shape) and crater-like imprint (lower right) due to fungus-like protrusion from the uterine epithelium (round shape). ×7,000.

induced both the increase in TAT activity in cell cultures along with the synthesis of a specific cell surface adhesive factor with the use of steroid hormones. Holmes and Dickson[3] proposed that these relationships existed in mouse blastocysts after demonstrating that, during the estrogen-induction of implantation, TAT activity increases in the trophoblast cells concomitant with changes in the electrochemical properties of the cell surface coat. Adding to this chain of action, Sahib, Jost, and Jost[26] recently induced TAT activity directly with cyclic AMP in cultured hepatoma cells, while Johnson and Pastan[27] also using cyclic AMP were able to increase the adhesion of fibroblasts to a substratum. All of these findings demonstrate a close relationship between cell surface adhesive properties, steroid hormones, cyclic AMP, and TAT, and all appear to function in the blastocyst during estrogen-induced activation.

The elimination of the inducing hormone by treatment with the second messenger, cyclic AMP, to achieve a similar result should be possible in this first and second messenger system. Indeed, this has been demonstrated clearly by increased incorporation of tritiated uridine into RNA synthesis in rat blastocysts induced by either estrogen[13] or cyclic AMP.[16] Furthermore, the changes in surface morphology of trophoblast cells accompanying blastocyst activation as seen by SEM now have been induced by either estrogen[7, 8] or cyclic AMP, as in the present results. Both morphologic and biochemical changes have been induced by cyclic AMP in cultured fetal rat brain cells.[28]

Intraperitoneal cyclic AMP appears to have a contraceptive effect on normal reproductive processes also. Ryan and Coronel[29] found that daily intraperitoneal injections of 5 mg. of cyclic AMP in normal female mice blocked the occurrence of pregnancy. Similar intraperitoneal treatment of mice with 5 and 10 mg. of cyclic AMP in the present results had no SEM activating effect, thus being consistent with the findings of Ryan and Coronel. To elucidate this contraceptive mechanism, Pearce and McClurg[30] conducted a histologic survey of the adrenal glands and reproductive organs after intraperitoneal cyclic AMP. They concluded that the contraceptive effect was mediated by changes in the ovaries, uterus, and vagina, indicative of a divergent hormonal effect. The activation effect induced by estrogen on the trophoblast cell surface was not present on blastocysts from mice

treated with intraperitoneal cyclic AMP, as mentioned above. In the present work, cyclic AMP activation of blastocysts apparently can be achieved only by local intraluminal administration of the nucleotide. Moreover, such a local intraluminal instillation of cyclic AMP in saline seems to mimic the effect of several contraceptive antiestrogens. Both produce estrogen-like activation of the blastocyst up to a point and later impede normal blastocyst proliferation and invasion of the stroma.[8] It seems that intraluminal cyclic AMP in saline is not enough to bring about a total estrogen effect, only the early changes. In this situation it has the effect on the blastocyst of an impeded estrogen.

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Radiation exposure of ovaries during hysterosalpingography

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Radiation exposure to an area approximating the location of the ovaries was measured in 29 patients undergoing hysterosalpingography. A thermoluminescent crystal technique was used for some patients; a pocket dosimeter placed into the upper vagina was used for additional patients. Radiation dosage varied from 75 to 550 millirads. The weight of the patient did not affect the amount of radiation delivered to the gonads. The duration of fluroscopic time was the major factor increasing dosage. A discussion of hysterosalpingographic technique and suggestions for decreasing gonadal irradiation during hysterosalpingography are included.

THOUGH CONTROVERSIAL, there is evidence to indicate that genetic mutation may be induced by less than a single roentgen of irradiation.[1-4]

Muller,[5] the Nobel prize geneticist, states in regard to irradiation of the gonads that there is "no dose so small as to give no mutations at all; each individual ionization and probably each activation of an atom carries its definite chance of producing a mutation." On the average, each newly mutated gene, no matter how small the detriment it occasions, eventually takes its toll in the form of making a major contribution to the extinction of the line of descent.

Schwartz[6] found that the radiosensitivity of the recently fertilized ovum is greatest in the gastrula and morula stages and that it decreases gradually during fetal life. The evidence cited implies that prefertilization radiation or postfertilization exposure may have detrimental effects.[7, 8] Radiation damage to the germ cells is cumulative without regard to time. A mutation need not be a monstrosity, but it chances adding to the load of inherited human ills. High-level dosage, of course can result in sterility.[1]

With the advent of image-intensified fluoroscopy and cinematography, radiation exposure to the ovaries could have been decreased. Unfortunately, these techniques have been so used as to increase radiation exposure manyfold.[9, 10]

The aim of the current investigation is to determine if radiation exposure to the ovaries can be decreased during hysterosalpingography. We have approached this question by measuring the ovarian radiation exposure during hysterosalpingography (HSG) with fluoroscopy.

Material and methods

All the patients included in this study were undergoing either infertility investigation or hysterosalpingography following tubal sterilization (HSG follow-up of tubal sterilization is a separate study).

A Foley catheter technique[11, 12] was used for hysterosalpingography.

Parekh and associates[11] recommended this method and we confirm its convenience as long as uterine manipulation is not required. In the latter event, a suction cannula is used rather than the Foley catheter.[13]

After draping and insertion of a Graves speculum, the cervix and vagina were painted with an antiseptic solution. A No. 10 or 12 Foley catheter was introduced steadily into the uterus with the help of a ring or uterine forceps. The bulb of the Foley was distended with 2 to 4 ml. of air.

Radiation exposure was measured with either of the following two devices applied as close to the ovaries as possible:

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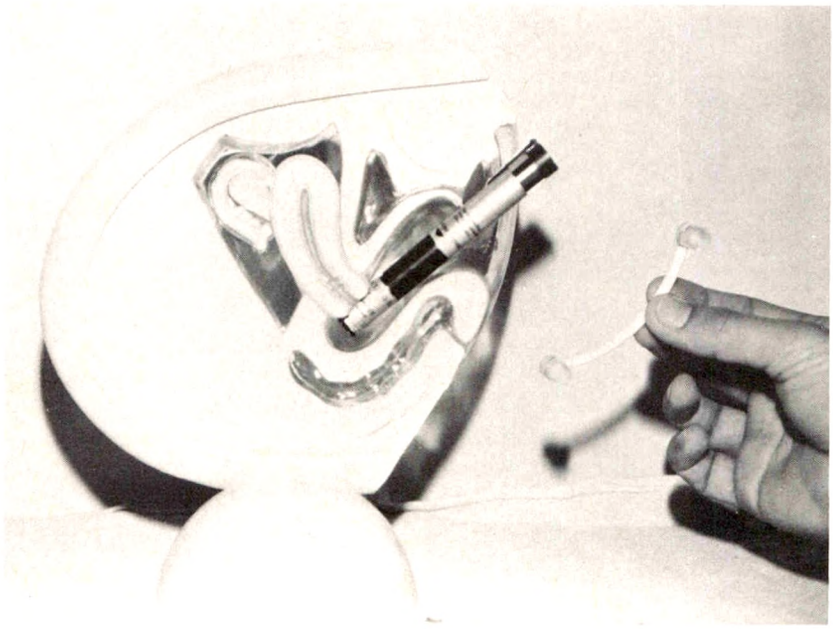


Fig. 1. Devices used for measuring radiation exposure to ovaries. Left, pocket dosimeter in model vagina; right, diaphragm device.

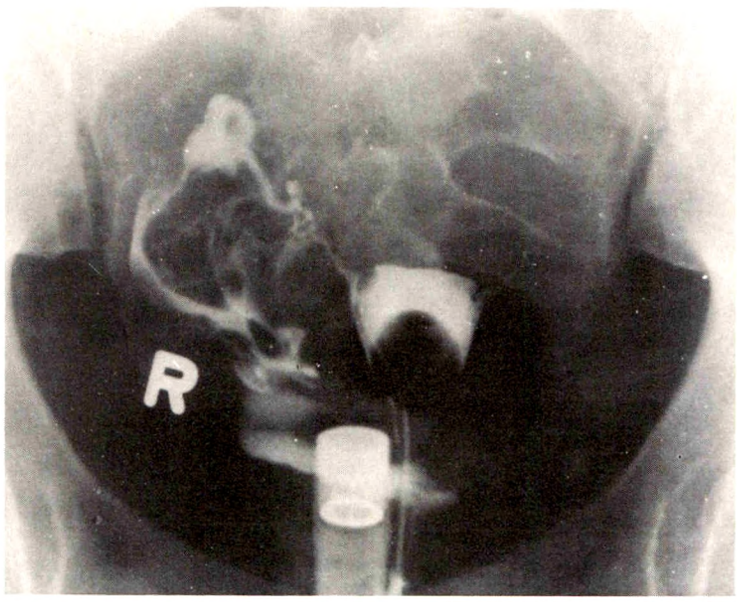


Fig. 2. Hysterosalpingography with the dosimeter in the vagina.

Thermoluminescent crystals (TLD principle) carried by small containers at the ends of an arc made from the curvature of a contraceptive diaphragm.[14, 15] The device was inserted into the vagina so as to place the crystals in the lateral fornices, thus measuring the radiation exposure in the vicinity of each ovary (Fig. 1). We were unable to continue using this device because of nonavailability of the dose-reading instrument.

An approximation of the irradiation exposure of the ovaries was obtained by a pocket dosimeter (Victoreen chamber principle).[15]

The dosimeter was placed in an empty surgical glove and inserted into the vagina so that the chamber was deeply placed in the posterior fornix. A hysterosalpingogram was performed, using fluoroscopy intermittently with minimum exposure time. All the procedures were carried out by the same operator, using the same x-ray machine (Westinghouse), with KVP kept

Table I. Detailed data in 29 patients

	Fluoroscopic time (sec.)	*Tube/film distance (in.)*	*Radiation amount (m.R.)*	*Weight (lb.)*	*Exposures*	*Fluoroscopic view*	*Indications*
1.	45	32	290	126	2	5	Post tubal coagulation
2.	46	29	333	170	2	3	Post tubal coagulation
3.	50	30	410	160	3	7	Post tubal coagulation
4.	20	33	70	127	2	2	Post tubal coagulation
5.	20	35	85	154	2	2	Post tubal coagulation
6.	22	33	75	139	2	2	Post tubal coagulation
7.	30	33	115	143	2	3	Post tubal coagulation
8.	35	32	150	174	2	3	Post tubal coagulation
9.	55	33	400	112	3	3	Post tubal coagulation
10.	60	34½	510	192	3	10	Post tubal coagulation
11.	50	33½	400	225	3	5	Post tubal coagulation
12.	48	32½	330	140	3	6	Post tubal coagulation
13.	50	30½	320	130	3	6	Post tubal coagulation
14.	50	33	400	129	3	4	Post tubal coagulation
15.	25	31	118	120	2	3	Post tubal coagulation
16.	55	29	400	92	2	4	Post tubal coagulation
17.	27	28	100	130	1	5	Post tubal coagulation
18.	65	32	550	104	2	6	Post tubal coagulation
19.	45	31	380	120	2	4	Post tubal coagulation
20.	47	31	480	160	2	3	Post tubal coagulation
21.	25	31	125	120	2	3	Post tubal coagulation
22.	36	29	210	110	2	3	Post tubal coagulation
23.	38	29½	225	138	2	3	Post tubal coagulation
24.	35	30½	160	142	2	3	Post tubal coagulation
25.	25	34	100	110	2	2	Post tubal coagulation
26.	25	33½	200	188	2	3	Post tubal coagulation
27.	42	33	270	100	2	4	Diagnostic
28.	45	33	113	300	2	4	Diagnostic
29.	30	35½	200	209	3	4	Diagnostic

constant at 100. Fig. 2 is a hysterosalpingogram with the dosimeter in place. A No. 10 Foley catheter is in the uterine cavity. Fig. 3 is a hysterosalpingograph with two dosimeters in the vaginal canal. A second dosimeter with a finer scale was used to determine the accuracy of the original device.

Results

Table I details the information noted for each patient. The total number of patients was 29; 26 were poststerilization procedures and three were indicated for diagnostic purposes. It will be noted that the weight of the patient was not related to the amount of radiation exposure. Radiation dosage increased with the number of fluoroscopic views taken as well as the duration of fluoroscopic exposure. The tube/film distance remained as constant as possible. The radiation exposure of the gonads was identical for diagnostic and poststerilization hysterosalpingography.

Table II summarizes the data derived from this study.

Comment

Cooper and associates,[16, 17] measuring the amount of radiation delivered to the gonads by placing an ionizing chamber in a female cadaver close to the ovaries during HSG, found a mean value of 322 mrad. Martin[18] found a value of 2,500 mrad. measured from the level of the skin. Berman and Sonnenblick[19] used an intravaginal instrument to measure the radiation dose delivered to the gonads and found it to be 3.22 rads for six films. All the above series were carried out without the use of fluoroscopy. Figures as high as 27 roentgens for HSG are also reported.[19, 20]

Shirley,[14] measuring the gonadal dosage with two plane film techniques, reported 130 mrad. With his careful use of fluoroscopy, the dosage was 1,000 to 1,500 mrad; Parekh and co-workers[11] found it to be 525 mrad. with three exposures, with fluoroscopy, 231.5 and 365.7 mrad., respectively. Thus, there is little difference between our findings and those of Parekh and Arronett, perhaps because the Foley catheter technique was used in both series. DeVeechi[10] has shown that during roentgen cinematography the ovaries receive about 2 rads per minute. Schwartz[6] has shown that different diagnostic x-ray machines may deliver 60 to 200 rads per minute; with cobalt treatment the dose is of the order of 10 to 50 rads per minute and with radium it is approximately 0.1 to 0.2 rads per minute. Schwartz further measured the

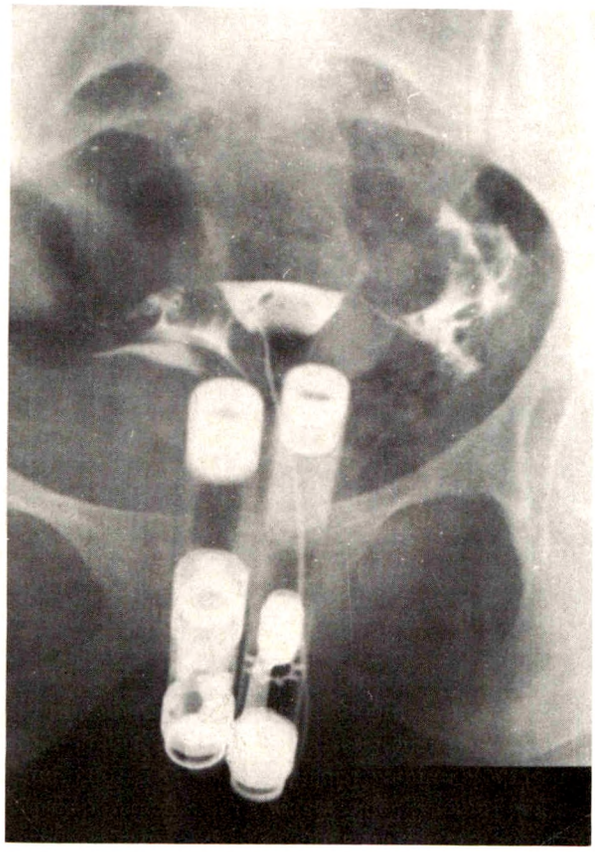


Fig. 3. Hysterosalpingography with two dosimeters in the vaginal cavity.

radiation given to the ovaries during such routine diagnostic procedures as intravenous pyelography, barium enema, and gastrointestinal series, at a total of 11 rads, higher than the maximum of 5 rads recommended radiation by the International Commission on Radiation Protection.[3] These figures represent quite high radiation exposures, and should be kept in mind

Table II. Summary of radiation data

Maximum Radiation (m.R.)	*Fluoroscopic view*	*Fluoroscopic time*
Maximum: Radiation 550	10	65
Minimum: Radiation 70	2	20

Average radiation with two exposures and fluoroscopy, 231.5 m.R.

Average radiation with three exposures and fluoroscopy, 365.7 m.R.

Average fluoroscopy time, 39.1 sec.

Average tube/film distance, 31.9 in.

Average fluoroscopic views, 4 (approximately).

Mean radiation for diagnostic HSG, 194 m.R.

before the decision is made to undertake a procedure on a fertile patient.

We feel that the current study confirms that (1) radiation exposure during hysterosalpingography can be decreased while retaining the efficacy of the procedure, and (2) hysterosalpingography can be simplified by the use of a Foley catheter technique.

Radiologic procedures involving gonad exposure, when indicated, should be done in the proliferative phase of the menstrual cycle, and radiologic exposure should be kept to a minimum.

We wish to thank Douglas M. Haynes, M.D., Professor of Obstetrics and Gynecology, and Curtis P. Sigdestad, M.D., Chief of Radiation Biology, for their critical review of the paper. We are indebted to John T. Queenan, M.D., Professor and Chairman, Department of Obstetrics and Gynecology, University of Louisville, for his encouragement in this project. Our appreciation to Mr. Ahren Jacobson, Radiation Physicist, for his technical advice and to the X-ray Department personnel for their cooperation in carrying out this study.

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Varices of Pregnancy

Impending motherhood is a time for great expectation, and time for increased risk of varices in the lower extremities, especially with a second or subsequent pregnancy. The discomfort and disfigurement of varices are an unwelcome and perhaps unnecessary intrusion upon these special moments. Yet, increased intra-abdominal pressure, expanded blood volume and decreased physical activity may come to sharp focus during pregnancy predisposing to varices of the lower extremities.

Naturally, varicose veins become more severe in subsequent pregnancies and may reach the critical stage. Temporary valve incompetence associated with chronic venous distention lays the groundwork for vascular impairment of a very permanent nature, perhaps varicosities of the lower extremities for life.

A Jobst Pregnancy support applies external counter-pressures that oppose excessive venous pressures during pregnancy. Fatigue and discomfort are relieved. Edema is alleviated.[1] A Jobst support worn during the critical months of gestation, may prevent permanent damage to distended veins.[2]

Thompson reports, "We have preferred the Jobst stocking in our clinic. An elastic leotard produced by this company is particularly useful for varicose veins during pregnancy. Most patients are pleasantly surprised by the comforting support this garment gives."[3]



Write for the Jobst Program of Treatment for Varicosities Attendant to Pregnancy. You will receive a collection of clinical reprints on the subject and a full color, twelve-page monograph and bibliography describing the physiological fluid dynamics of external elastic compression. You might also like a twenty-eight-page patient education booklet, edited by a prominent obstetrician, entitled: Answering Your Questions About Varicose Veins During Pregnancy.

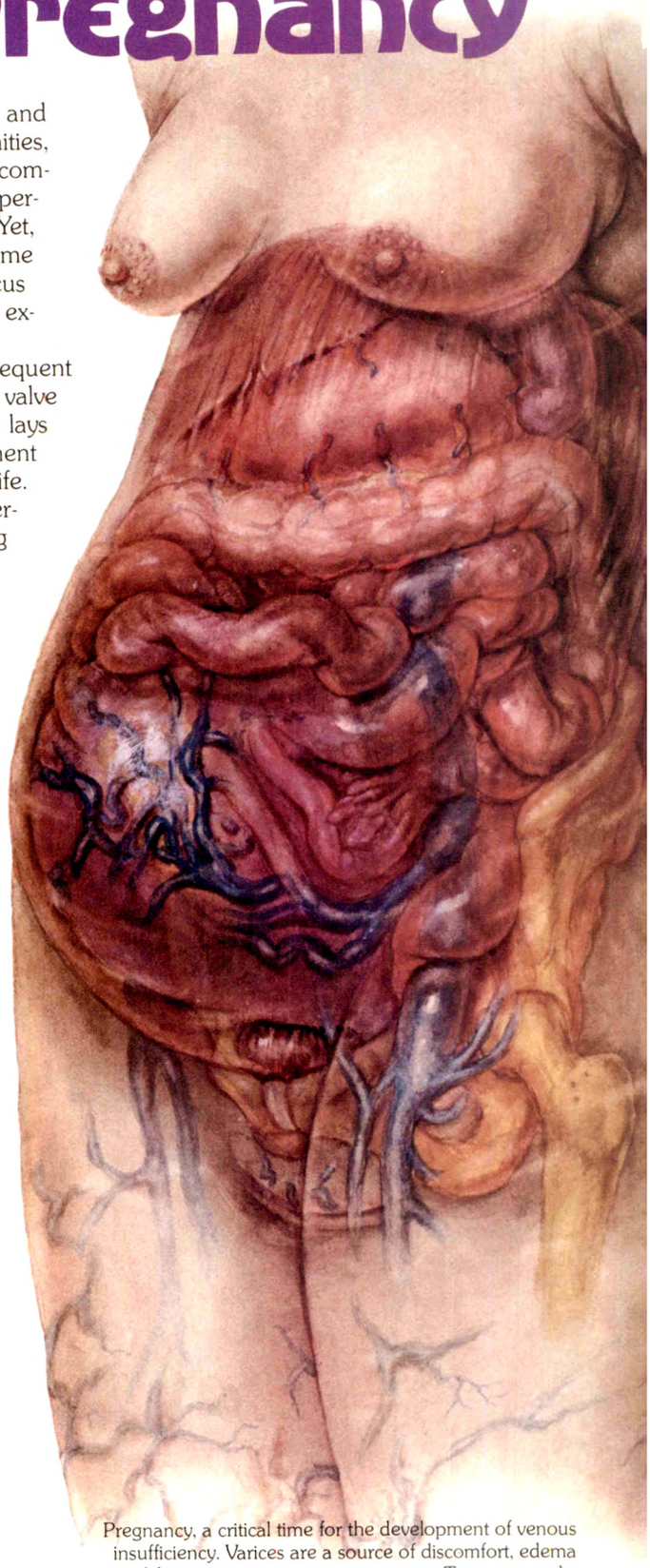
Varices of Pregnancy?
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Pregnancy, a critical time for the development of venous insufficiency. Varices are a source of discomfort, edema and fatigue to many pregnant women. Temporary valve incompetence associated with chronic venous distention lays the groundwork for vascular impairment of a very permanent nature. Perhaps, disfigurement for life.

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Microbiologic aspects of fertility...

The greatest potential for bacterial infection in the female genital tract exists during the fertile years. A large proportion of the potentially pathogenic microflora are anaerobic—particularly bacteroides and anaerobic streptococci. Isolation and identification present special problems.

"Pseudo-maturity"

Sterile at the time of birth, the female genitalia begin to develop an abundant bacterial flora within hours. But this maternally influenced flora, composed largely of the familiar gram-positive lactobacillus known as Döderlein's bacillus, disappears in just a few weeks. The vaginal secretions revert toward alkalinity and remain alkaline until puberty. A varied flora of staphylococci, streptococci (except pyogenes), coliforms and diphtheroid bacilli is present.

The healthy mature female

With the onset of menstruation, conditions within the vagina change appreciably. For example, the glycogen content of the vaginal epithelium is markedly increased under the influence of ovarian hormones. This, in turn, encourages the proliferation of Döderlein's bacilli. These lactobacilli create an acid environment which limits the growth of potential pathogens, and the role of lactobacilli in keeping the microflora in balance is vital throughout a woman's reproductive years.

The normal flora—anaerobes predominate

Certain other bacteria are also seen regularly in the vagina. With today's improved methods of culturing, it has been demonstrated that a large proportion of these bacteria are anaerobic—notably bacteroides and anaerobic streptococci. Other microorganisms that may be observed are the *Escherichia coli/Enterobacter aerogenes* group of coliforms, the anaerobic micrococci, group B streptococci, enterococci, diphtheroids, and *Hemophilus vaginalis.* The presence of any of these microorganisms may or may not be a matter of etiologic importance.

Disturbing influences—balance upset

Not all gynecologic infections are of endogenous origin. Changes in hormonal balance—for example, during pregnancy—nearly always influence the microbiological balance. Both



the resident bacterial population and potential invading pathogens may be affected. Either may be responsible for the occurrence of postpartum infections, postabortal infections, posthysterectomy infections, and the infections that occur concurrently with neoplastic disease. As pathogens ascend the reproductive tract from vagina to uterus to tubes, the risk to the patient is usually enhanced.

Forms of infection

Gynecologists and surgeons alike are well acquainted with such infections: abscesses (whether tuboovarian, pelvic, vaginal, vulval, or Bartholin's gland infection), puerperal sepsis, salpingitis, endometritis, peritonitis of gynecologic origin, vaginal-cuff infection, chronic pelvic inflammatory disease—with each, the threat of possible septicemia. The need for immediate surgical intervention may be controversial, but certain of these infections often represent true gynecologic emergencies.

The problems anaerobes pose...culturing Improved culturing techniques have demonstrated that anaerobes—especially bacteroides and anaerobic streptococci—inhabit the vagina, as well as more remote genital areas. It has been reported that these usually foul-odor-producing anaerobic organisms may be isolated in three out of four cases of infections of the genital tract. In one series of patients, anaerobes alone were cultured in 37 percent of the cases. Many studies have shown that bacteroides are by far the most common of these organisms, but anaerobes such as peptococci, peptostreptococci and other anaerobic streptococci are also frequently observed. Mixed infections with multiple anaerobic species are not unusual.

Today many physicians suspect that the techniques of obtaining or incubating the culture may be at fault if no anaerobes at all are observed.

A new appreciation of pathogenicity Only recently has it been realized that anaerobes other than clostridia are pathogens of major significance in serious infections of the female genital tract. Exacting methods of culturing have helped to establish this. One investigation has shown that 44 percent of all anaerobic isolates obtained from a series of 400 patients of all types, not just gynecologic, were associated with infection. Bacteroides and anaerobic streptococci were the most prevalent, with clostridia occurring less frequently.

Whenever these organisms are seen on repeated occasions by Gram stain or isolated by culture—whether alone or combined with other bacteria—they should not be ignored.

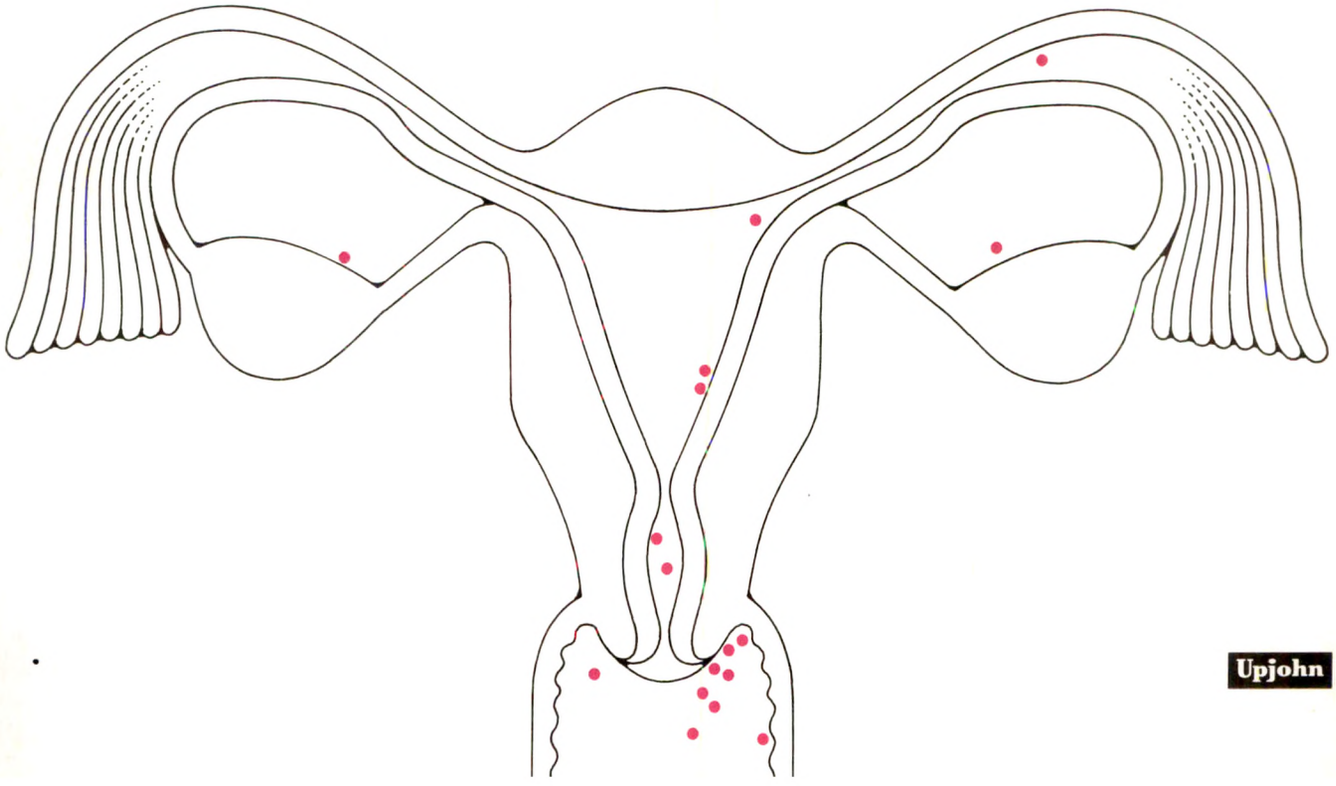
Therapeutic measures

Selection of the most appropriate antibiotic—or antibiotics—is still a problem for the gynecologist in many instances, since many of the infections he deals with cannot be etiologically defined when first observed. Nevertheless, the seriousness of his patient's condition may demand immediate attention. Because there are numerous antibiotics effective against just certain potential pathogens, the physician's problem has been intensified. And for one aspect of his problem—anaerobic infections—there has been no easy answer.

Many gynecologists believe that, of all infections, these present one of today's most serious challenges.

For information on ONE ANTIBIOTIC OF SIGNIFICANCE in the treatment of serious anaerobic infections of the female reproductive tract, please turn page.

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Therapeutic considerations...

Cleocin® Phosphate (clindamycin phosphate injection) has proved to be effective against susceptible anaerobic organisms (whether they are anaerobic cocci or anaerobic gram-positive or gram-negative rods). Specifically its activity has been established in serious infections of the female pelvis and genital tract such as endometritis, nongonococcal tubo-ovarian abscess, pelvic cellulitis, and postsurgical vaginal-cuff infection caused by susceptible strains of bacteroides, fusobacteria, peptococci, peptostreptococci, microaerophilic streptococci, or clostridia.* Furthermore, Cleocin PO_4 has proved successful in septicemia and in such nongynecologic conditions as serious intra-abdominal infections (peritonitis, intra-abdominal abscess), serious respiratory-tract infections (empyema, anaerobic pneumonitis, lung abscess), and serious infections of skin and soft-tissue caused by susceptible anaerobic organisms.

Since disc susceptibility testing with current methods is not reliable for anaerobes, *in vitro* dilution methods should be used to determine susceptibility to clindamycin.

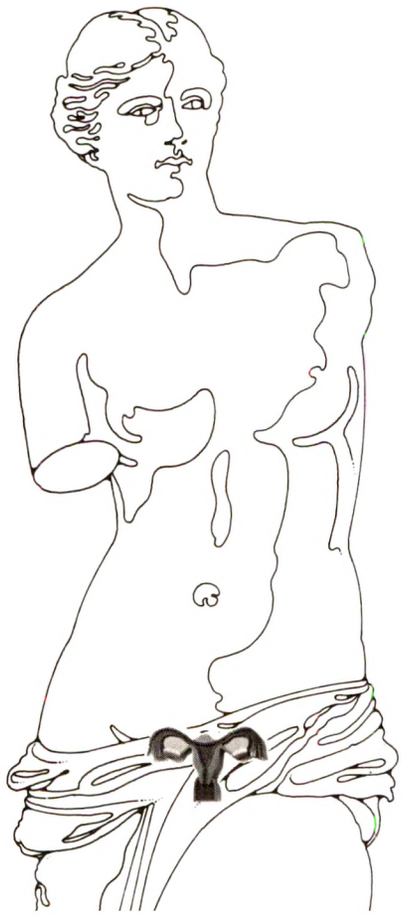
Also of value in serious gram-positive infections The use of Cleocin PO_4 in serious nongynecologic infections caused by susceptible gram-positive organisms has been extensively investigated both bacteriologically and clinically. Because Cleocin PO_4 is a parenteral antibiotic, emphasis has been on evaluation of the drug in serious infections, and the appropriate patients for Cleocin PO_4 are those who are hospitalized with serious illnesses.

Diagnoses have included septicemia and septic shock, acute peritonitis, staphylococcal pneumonia, and massive wound infection. In many cases the infection was secondary to severe accidental trauma, major surgery, or debilitating disease.

Cleocin PO_4 has produced excellent results when the pathogens in these serious infections proved to be staphylococci, streptococci (except enterococci), or pneumococci. With rare exceptions, the organisms proved susceptible *in vitro* and were successfully eradicated from the clinical lesions, regardless of the severity of the infection. Its use in these infections should be reserved for penicillin-allergic patients for whom, in the judgment of the physician, a penicillin is inappropriate.

Other therapeutic considerations:

1) Cleocin PO_4 is clinically effective in serious infections caused by penicillin-resistant or penicillinase-producing staphylococci. However, staphylococcal strains resistant to clindamycin have been encountered, and susceptibility testing should be performed.



2) Cleocin is useful in patients who are sensitive to penicillin. Rare cases of sensitivity to Cleocin resulting in maculopapular rash and urticaria have been reported; as with all antibiotics, it should be used cautiously in atopic patients. Serious anaphylactoid reactions require emergency treatment with epinephrine. Oxygen and intravenous corticosteroids should also be administered as indicated. However, no actual cross-antigenicity with other antibiotics has been demonstrated, *except with lincomycin.*

Because of these two benefits, Cleocin PO_4 can fill a need both for penicillin-sensitive patients and those harboring penicillin-resistant organisms.

Cleocin PO_4 should be reserved for serious infections where less toxic antimicrobial agents are inappropriate, as described in the Indications Section of the brief summary on the following page.

WARNINGS: (See WARNING box on following page.)

If significant diarrhea or colitis occurs during therapy, this antibiotic should be discontinued. (See WARNING box and summary of prescribing information on following page.

Safety for use in pregnancy has not been established.

*Clostridia are more resistant than most anaerobes to clindamycin. Some species, e.g., *C. sporogenes* and *C. tertium*, are frequently resistant to clindamycin.

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WARNING

Clindamycin can cause severe colitis which may end fatally. Therefore, it should be reserved for serious infections where less toxic antimicrobial agents are inappropriate, as described in the Indications Section. It should not be used in patients with nonbacterial infections, such as most upper respiratory tract infections. The colitis is usually characterized by severe, persistent diarrhea and severe abdominal cramps and may be associated with the passage of blood and mucus. Endoscopic examination may reveal pseudomembranous colitis.

When significant diarrhea occurs, the drug should be discontinued or, if necessary, continued only with close observation of the patient. Large bowel endoscopy has been recommended.

Antiperistaltic agents such as opiates and diphenoxylate with atropine (Lomotil®) may prolong and/or worsen the condition.

Diarrhea, colitis, and pseudomembranous colitis have been observed to begin up to several weeks following cessation of therapy with clindamycin.

Each ml contains:

clindamycin phosphate equivalent to. . . . . 150 mg clindamycin base

benzyl alcohol. . . . . 5 mg

disodium edetate. . . . . 0.5 mg

water for injection. . . . . qs

When necessary, pH adjusted with NaOH and/or HCl.

Indications: Clindamycin is indicated in the treatment of serious infections caused by susceptible anaerobic bacteria.

Clindamycin is also indicated in the treatment of serious infections due to susceptible strains of streptococci, pneumococci, and staphylococci. Its use should be reserved for penicillin-allergic patients or other patients for whom, in the judgment of the physician, a penicillin is inappropriate. Because of the risk of colitis, as described in the WARNING box, before selecting clindamycin the physician should consider the nature of the infection and the suitability of less toxic alternatives (e.g., erythromycin).

Anaerobes: Serious respiratory tract infections such as empyema, anaerobic pneumonitis and lung abscess; serious skin and soft tissue infections; septicemia; intra-abdominal infections such as peritonitis and intra-abdominal abscess (typically resulting from anaerobic organisms resident in the normal gastrointestinal tract); infections of the female pelvis and genital tract such as endometritis, nongonococcal tubo-ovarian abscess, pelvic cellulitis and postsurgical vaginal cuff infection.

Streptococci: Serious respiratory tract infections; serious skin and soft tissue infections; septicemia.

Staphylococci: Serious respiratory tract infections; serious skin and soft tissue infections; septicemia; acute hematogenous osteomyelitis.

Pneumococci: Serious respiratory tract infections.

Adjunctive Therapy: In the surgical treatment of chronic bone and joint infections due to susceptible organisms.

Indicated surgical procedures should be performed in conjunction with antibiotic therapy.

Bacteriologic studies should be performed to determine the causative organisms and their susceptibility to clindamycin.

Contraindications: History of hypersensitivity to clindamycin or lincomycin.

Warnings: (See WARNING box.) Mild cases of colitis may respond to drug discontinuance alone. Moderate to severe cases should be managed promptly with fluid, electrolyte and protein supplementation as indicated. Systemic corticoids and corticoid retention enemas may help relieve the colitis. Other causes of colitis should also be considered.

A careful inquiry should be made concerning previous sensitivities to drugs and other allergens. Antagonism has been demonstrated between clindamycin and erythromycin *in vitro*. Because of possible clinical significance, these two drugs should not be administered concurrently.

Usage in Pregnancy: Safety for use in pregnancy has not been established.

Usage in Newborns and Infants: When clindamycin phosphate is administered to newborns and infants, appropriate monitoring of organ system functions is desirable.

Usage in Meningitis: Since clindamycin does not diffuse adequately into the cerebrospinal fluid, the drug should not be used in meningitis.

SERIOUS ANAPHYLACTOID REACTIONS REQUIRE IMMEDIATE EMERGENCY TREATMENT WITH EPINEPHRINE. OXYGEN AND INTRAVENOUS CORTICOSTEROIDS SHOULD ALSO BE ADMINISTERED AS INDICATED.

Precautions: Review of experience to date suggests that a subgroup of older patients with associated severe illness may tolerate diarrhea less well. When clindamycin is indicated in these patients, they should be carefully monitored for change in bowel frequency. Prescribe with caution in individuals with a history of gastrointestinal disease, particularly colitis.



Do not inject intravenously as an undiluted bolus; infuse as directed in package insert. Indicated surgical procedures should be performed in conjunction with therapy. Patients with very severe renal disease and/or very severe hepatic disease accompanied by severe metabolic aberrations should be dosed with caution and serum clindamycin levels monitored during high dose therapy.

Prescribe with caution in atopic individuals. During prolonged therapy, periodic liver and kidney function tests and blood counts should be performed. Use may result in overgrowth of non-susceptible organisms, particularly yeast. Should superinfection occur, adjust therapy as clinical situation dictates. Clindamycin has been shown to have neuromuscular blocking properties that may enhance the action of other neuromuscular blocking agents. Use with caution in patients receiving such agents.

Adverse Reactions: *Gastrointestinal:* Abdominal pain, nausea, vomiting and diarrhea. (See WARNING box.) *Hypersensitivity Reactions:* Maculopapular rash and urticaria have been observed. Generalized mild to moderate morbilliform-like skin rashes are the most frequently reported of all adverse reactions. Rare instances of erythema multiforme, some resembling Stevens-Johnson syndrome, have been associated with clindamycin. A few cases of anaphylactoid reactions have been reported. If a hypersensitivity reaction occurs, the drug should be discontinued. The usual agents (epinephrine, corticosteroids, antihistamines) should be available for emergency treatment of serious reactions. *Liver:* Jaundice and abnormalities in liver function tests have been observed during clindamycin therapy. *Hematopoietic:* Neutropenia, eosinophilia, agranulocytosis and thrombocytopenia have been reported; no direct etiologic relationship to concurrent clindamycin therapy has been made.

Local Reactions: Pain, induration and sterile abscess have been reported after intramuscular injection and thrombophlebitis after intravenous infusion. Reactions can be minimized or avoided by giving deep intramuscular injections and avoiding prolonged use of indwelling intravenous catheters.

How Supplied: Available as sterile solution with each ml containing clindamycin phosphate equivalent to 150 mg clindamycin base. Ampoules of 2 and 4 ml.

Caution: Federal law prohibits dispensing without prescription.

For additional product information see your Upjohn representative.

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An effective low-dose oral contraceptive

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Ortho-Novum 1/50 □ 21

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Each tablet contains 1 mg norethindrone and 0.05 mg mestranol.

A sensible way to start a woman on oral contraceptives is with an effective, low-dose combination like Ortho-Novum 1/50 □ 21. This combination offers a low dose of estrogen, 50 mcg of mestranol, and a low dose of progestin, 1 mg norethindrone, which can fulfill your contraceptive requirements and her contraceptive needs.

Ortho-Novum 1/50 □ 21 is usually a well-tolerated† combination; thus, in clinical studies, there is a low drug-related drop-out rate.

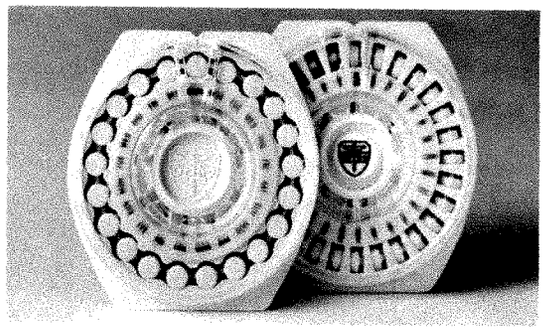
Add to this the simplicity of the dosage regimen — three weeks on and one week off — and the unique Dialpak* Tablet Dispenser, which helps reduce the chance of error or missed doses, and you have an effective combination that fits very nicely into their life-style...and their wor

†Serious as well as minor conditions have been reporte following the use of oral contraceptives. These conditi include thromboembolic disease. The physician should alert to the earliest manifestations of any symptoms of serious disease and discontinue oral contraceptive the when appropriate. The physician should be fully awar of the complete Prescribing Information for this produ

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See prescribing information on following page.

an effective low-dose oral contraceptive



Ortho-Novum 1/50□21 and 28

TRADEMARK

Ortho-Novum 1/50□21 and Ortho-Novum 1/50□28

Description: Each ORTHO-NOVUM 1/50□21 Tablet contains 1 mg of the progestational substance, norethindrone, together with 0.05 mg of the estrogenic compound, mestranol.

Each yellow ORTHO-NOVUM 1/50□28 Tablet contains 1 mg of the potent progestational substance, norethindrone, together with 0.05 mg of the estrogenic compound, mestranol. Each green tablet contains inert ingredients.

Actions: ORTHO-NOVUM 1/50□21 and ORTHO-NOVUM 1/50□28 act through the mechanism of gonadotrophin suppression, i.e., through the estrogenic and progestational actions of the ingredients. Alterations in cervical mucus and in the endometrium may also contribute to the efficacy of ORTHO-NOVUM 1/50□21 and ORTHO-NOVUM 1/50□28.

Special note: Oral contraceptives have been marketed in the United States since 1960. Reported pregnancy rates vary from product to product. The effectiveness of the sequential products appears to be somewhat lower than that of the combination products. Both types provide almost completely effective contraception.

The physician should consider a thorough discussion with each patient about the prescribing information, risks and benefits associated with oral contraceptives, taking into account various factors such as age and other physical conditions.

An increased risk of thromboembolic disease associated with the use of hormonal contraceptives has now been shown in studies conducted in both Great Britain and the United States. Other risks, such as those of elevated blood pressure and reduced tolerance to carbohydrates, have been reported and appropriate tests should be conducted to monitor these during oral contraceptive therapy. Liver disease has also been reported, and the physician should be alert to its earliest manifestations.

Long-term administration of both natural and synthetic estrogens in subprimate animal species in multiples of the human dose increases the frequency for some animal carcinomas. These data cannot be transposed directly to man. The possible carcinogenicity due to the estrogens can neither be affirmed nor refuted at this time. Close clinical surveillance of all women taking oral contraceptives must be continued.

Indication: Contraception.

Contraindications: 1. Thrombophlebitis, thromboembolic disorders, cerebral vascular disease, myocardial infarction, or a past history of these conditions. 2. Markedly impaired liver function. 3. Known or suspected carcinoma of the breast. 4. Known or suspected estrogen-dependent neoplasia. 5. Undiagnosed abnormal genital bleeding. 6. Known or suspected pregnancy.

Warnings: 1. The physician should be alert to the earliest manifestations of thrombotic and thromboembolic disorders, thrombophlebitis, cerebrovascular disorders including hemorrhage, pulmonary embolism and retinal thrombosis. Should any of these occur or be suspected, the drug should be discontinued immediately.

Retrospective studies of morbidity and mortality in Great Britain and studies of morbidity in the United States have shown a statistically significant association between thrombophlebitis, pulmonary embolism and cerebral vascular disease, occlusive or hemorrhagic, and the use of oral contraceptives. There have been three principal studies in Great Britain[1-3] leading to these conclusions and three in this country.[4-7] The estimate of the relative risk of thromboembolism in the study by Vessey and Doll[3] was about sevenfold, while the United States studies found relative risks of 4.4 to 11, meaning that the users are several times as likely to undergo thromboembolic disease without evident cause as nonusers.

In May, 1974, the Royal College of General Practitioners in England[8] issued an interim report of its continuing large-scale prospective study comparing a user group to a nonuser group. This study in its interim analysis states, "A statistically significant higher rate of reporting of cerebrovascular accidents in Takers is evident, but the numbers are too small to justify an estimation of the degree of risk." The study also reported a higher incidence of superficial and deep vein thrombosis in users as compared to nonusers. The risk of superficial and deep vein thrombosis was reported to be lower in women using 50 mcg estrogen preparations.

The Sartwell study[4] indicated that the risk did not persist after discontinuation of administration. Both the Sartwell and the Royal College studies indicated that the degree of risk was not associated with duration of treatment.

In a collaborative American study[5,6] of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the relative risk of thrombotic stroke was 4.1 to 9.5 times greater in users than in nonusers. A comparable estimate for hemorrhagic stroke was 2.0.

None of the American studies was designed to evaluate a difference between products. However, the Sartwell study[4] suggested that there might be an increased risk of thromboembolic disease in users of sequential products.

Other retrospective studies[9,10] have reported an increased risk of post-surgery thromboembolic complications in oral contraceptive users. It has been recommended that therapy be discontinued at least one month prior to elective surgery.

In May of 1975 two separate retrospective case control studies relating to fatal and nonfatal myocardial infarction in users and nonusers of oral contraceptives were reported.[11,12] The studies estimate that the increased risk of hospital admission for myocardial infarction associated with the use of oral contraceptives as compared to nonusers is 2.7 times in the 30-39 age group and 5.7 times in the 40-44 age group; and the risk of death associated with the use of oral contraceptives as compared to nonusers was increased 2.8 times for users in the 30-39 age group and 4.7 times in the 40-44 age group. One of these studies reported that oral contraceptive users who had myocardial infarction were more likely to be heavy smokers and have certain additional risk factors, i.e., treated hypertension and diabetes, pre-eclamptic toxemia, obesity and type II hyperlipoproteinemia.

2. Discontinue oral contraceptive medication if there is: gradual or sudden partial or complete loss of vision; proptosis or diplopia; onset or aggravation of migraine or development of headache of a new pattern which is recurrent, persistent or severe; papilledema; or any evidence of retinal vascular lesions.

3. Fetal abnormalities have been reported to occur in the offspring of women who have taken progestogens and/or estrogens during pregnancy.[13,14] The safety of ORTHO-NOVUM 1/50□21 or ORTHO-NOVUM 1/50□28 in pregnancy has not been demonstrated. Pregnancy should be ruled out before initiating or continuing the contraceptive regimen. Pregnancy should always be considered if withdrawal bleeding does not occur.

4. A small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The long-range effect to the nursing infant cannot be determined at this time.

5. Hepatic lesions (adenomas, hepatomas, hamartomas, regenerating nodules, etc.), occasionally fatal, have been reported in women on oral contraceptives. Such lesions may present as an abdominal mass or with the signs and symptoms of an acute abdomen. These lesions should be considered if the patient has abdominal pain or evidence of intra-abdominal bleeding. This has been reported in short-term as well as long-term users of oral contraceptives.

Precautions: 1. A thorough history and physical examination should be performed before prescribing oral contraceptives and periodically during their administration and should include special reference to breasts and pelvic organs, including Papanicolaou smear since estrogens have been known to produce tumors, some of them malignant, in five species of subprimate animals. 2. Endocrine and possibly liver function tests may be affected by treatment with ORTHO-NOVUM 1/50□21 or ORTHO-NOVUM 1/50□28. Therefore, if such tests are abnormal in a patient taking ORTHO-NOVUM 1/50□21 or ORTHO-NOVUM 1/50□28, it is recommended that they be repeated after the drug has been withdrawn for two months. 3. Under the influence of estrogen-progestogen preparations, pre-existing uterine fibromyomata may increase in size. 4. Because these agents may cause some degree of fluid retention, conditions which might be influenced by this factor, such as epilepsy, migraine, asthma, cardiac or renal dysfunction, require careful observation. 5. In breakthrough bleeding, and in all cases of irregular bleeding per vaginam, nonfunctional causes should be borne in mind. In undiagnosed bleeding per vaginam, adequate diagnostic measures are indicated. 6. Patients with a history of psychic depression should be carefully observed and the drug discontinued if the depression recurs to a serious degree. 7. Any possible influence of prolonged ORTHO-NOVUM 1/50□21 or ORTHO-NOVUM 1/50□28 therapy on pituitary, ovarian, adrenal, hepatic or uterine function awaits further study. 8. A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. The mechanism of this decrease is obscure. For this reason, diabetic patients should be carefully observed while receiving ORTHO-NOVUM 1/50□21 or ORTHO-NOVUM 1/50□28 therapy. 9. The age of the patient constitutes no absolute limiting factor, although treatment with ORTHO-NOVUM 1/50□21 or ORTHO-NOVUM 1/50□28 may mask the onset of the climacteric. 10. The pathologist should be advised of ORTHO-NOVUM 1/50□21 or ORTHO-NOVUM 1/50□28 therapy when relevant specimens are submitted. 11. Susceptible women may experience an increase in blood pressure following administration of contraceptive steroids. 12. Cholestatic jaundice has been reported in users of oral contraceptives. If this occurs, ORTHO-NOVUM 1/50□21 or ORTHO-NOVUM 1/50□28 should be discontinued. This condition is more likely to occur in patients who have experienced cholestatic jaundice of pregnancy. Patients with a history of cholestatic jaundice of pregnancy should be carefully observed during ORTHO-NOVUM 1/50□21 or ORTHO-NOVUM 1/50□28 therapy.

Adverse reactions observed in patients receiving oral contraceptives: A statistically significant association has been demonstrated between use of oral contraceptives and the following serious adverse reactions: thrombophlebitis, pulmonary embolism, cerebral thrombosis and hemorrhage, myocardial infarction, gallbladder disease.

Although available evidence is suggestive of an association, such a relationship has been neither confirmed nor refuted for the following serious adverse reactions: neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis; hepatic lesions with or without intra-abdominal bleeding.

The following adverse reactions are known to occur in patients receiving oral contraceptives: nausea, vomiting, gastrointestinal symptoms (such as abdominal cramps and bloating), breakthrough bleeding, spotting, change in menstrual flow, amenorrhea during and after treatment, edema, chloasma or melasma, which may persist, cholestatic jaundice, migraine, rash (allergic), mental depression, change in weight (increase or decrease), breast changes (tenderness, enlargement and secretion), changes in cervical erosion and cervical secretions, suppression of lactation when given immediately post-partum, rise in blood pressure in susceptible individuals.

Although the following adverse reactions have been reported in users of oral contraceptives, an association has been neither confirmed nor refuted: anovulation post-treatment, which tends to occur more frequently in women with a history of menstrual disorders, premenstrual-like syndrome, changes in libido, changes in appetite, cystitis-like syndrome, headache, intolerance to contact lenses, nervousness, dizziness, fatigue, backache, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruptions, itching, vaginitis.

The following laboratory results may be altered by the use of oral contraceptives: hepatic function—increased sulfobromophthalein retention and other tests; coagulation tests—increase in prothrombin, Factors VII, VIII, IX and X; decrease in anti-thrombin III; increase in platelet aggregability; thyroid function—increase in PBI, and butanol-extractable protein-bound iodine and decrease in T^3 uptake values; metyrapone test; pregnanediol determination.

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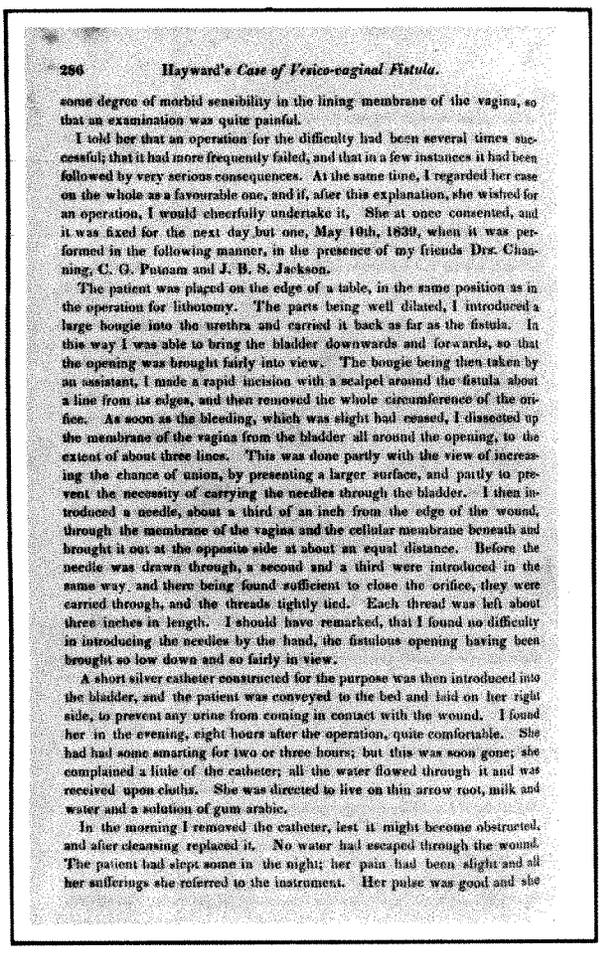
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Classic pages in
OBSTETRICS AND GYNECOLOGY
Lawrence D. Longo, M.D.

Case of vesico-vaginal fistula, successfully treated by an operation

GEORGE HAYWARD
American Journal of Medical Sciences, vol. 24, pp. 283-288, 1839



250 Hayward's *Case of Vesico-vaginal Fistula.*

some degree of morbid sensibility in the lining membrane of the vagina, so that an examination was quite painful.

I told her that an operation for the difficulty had been several times successful; that it had more frequently failed, and that in a few instances it had been followed by very serious consequences. At the same time, I regarded her case on the whole as a favourable one, and if, after this explanation, she wished for an operation, I would cheerfully undertake it. She at once consented, and it was fixed for the next day but one, May 10th, 1839, when it was performed in the following manner, in the presence of my friends Drs. Channing, C. G. Putnam and J. B. S. Jackson.

The patient was placed on the edge of a table, in the same position as in the operation for lithotomy. The parts being well dilated, I introduced a large bougie into the urethra and carried it back as far as the fistula. In this way I was able to bring the bladder downwards and forwards, so that the opening was brought fairly into view. The bougie being then taken by an assistant, I made a rapid incision with a scalpel around the fistula about a line from its edges, and then removed the whole circumference of the orifice. As soon as the bleeding, which was slight had ceased, I dissected up the membrane of the vagina from the bladder all around the opening, to the extent of about three lines. This was done partly with the view of increasing the chance of union, by presenting a larger surface, and partly to prevent the necessity of carrying the needles through the bladder. I then introduced a needle, about a third of an inch from the edge of the wound, through the membrane of the vagina and the cellular membrane beneath and brought it out at the opposite side at about an equal distance. Before the needle was drawn through, a second and a third were introduced in the same way, and there being found sufficient to close the orifice, they were carried through, and the threads tightly tied. Each thread was left about three inches in length. I should have remarked, that I found no difficulty in introducing the needles by the hand, the fistulous opening having been brought so low down and so fairly in view.

A short silver catheter constructed for the purpose was then introduced into the bladder, and the patient was conveyed to the bed and laid on her right side, to prevent any urine from coming in contact with the wound. I found her in the evening, eight hours after the operation, quite comfortable. She had had some smarting for two or three hours; but this was soon gone; she complained a little of the catheter; all the water flowed through it and was received upon cloths. She was directed to live on thin arrow root, milk and water and a solution of gum arabic.

In the morning I removed the catheter, lest it might become obstructed, and after cleansing replaced it. No water had escaped through the wound. The patient had slept some in the night; her pain had been slight and all her sufferings she referred to the instrument. Her pulse was good and she

The first report of successful closure of vesicovaginal fistula in America. Although the case of John P. Mettauer occurred the previous year (1838), it was not reported until the following year (Boston Med. Surg. J. 22: 154, 1840). In a later article, Hayward observed, regarding this first case, "I had never seen the operation done, till I did it myself, nor could I find any description of the mode which others had adopted, that was sufficiently clear and explicit to be of much service. I had, therefore, to take such a course as I thought safe, and at the same time likely to effect the object. . . ." The patient, age 34, had developed the fistula following delivery of her first child 15 years previously. Subsequently, she aborted 11 times. Hayward, Professor of Surgery at Harvard University and surgeon at the Massachusetts General Hospital, used a whalebone bougie inserted through the urethra to bring the fistulous opening into view, excised the cicatrix around the opening, widely dissected the bladder from the vagina, and closed the opening with three silk threads through the outer wall of the bladder (see left). A silver retention catheter was left in place for one week. Hayward concluded, "I have ventured to make these suggestions, which I do with great diffidence, with regard to the mode of operating, because there is no case in surgery in which a successful operation gives more complete relief . . . or relieves a greater amount of wretchedness, and because it is by no means settled what is the best mode of treating this distressing infirmity." In all, Hayward operated on nine patients (Boston Med. Surg. J. 44: 209, 1851). In three patients the results were entirely successful; four gained considerable relief but still had some incontinence, and two received no benefit.

CURRENT DEVELOPMENTS

Developments in mammography

JOHN N. WOLFE, M.D.

Detroit, Michigan

Mammography is presented for the primarily nonradiologic audience. A brief historical review calls attention to some of the milestones in mammography and how it has changed. Xeroradiography is discussed, as it is one of the newer developments and is rapidly gaining broad acceptance in the United States. Clinical applications are included, with a discussion of the various entities which the radiologist can identify on the mammogram. Although the mammographic examination can be extremely accurate in the hands of interested examiners, some carcinomas will not be identified; of these, some can be discovered by routine physical examination. If mammography is made to stand alone, without a physical examination, some women with breast cancer will not be identified and will not receive prompt, adequate treatment. Mammography is an adjunct and a complement to the physical examination. Breast cancer screening appears to be effective in finding small, nonpalpable tumors, many very early in their growth. Definitive evidence is now available that early diagnosis of breast cancer leads to prolonged survival. There is promise of further developments in the field of mammography.

RADIOGRAPHIC examination of the breast, or mammography, has a long history and one could characterize that history by dividing it into segments. The first part would be the era of early experimentation, which took place during the 1930's. The next era was one of limited clinical application of mammography and this lasted until the late 1950's. Beginning in the late 1950's and extending until the present time there has been an accelerated acceptance and utilization of this diagnostic examination for breast cancer detection. The present era is one of widespread application on a day-to-day clinical basis, trials in large-scale screening of asymptomatic women, and a rapidly accelerating technology with a constant search for new and better methods of either carrying out the examination or interpreting the images.

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This communication presents mammography in the following order: a brief historical review, newer developments in the technique of examination, including those being used in practice and those still experimental, breast cancer screening, and, finally, the development of criteria for assigning patients to risk categories for breast cancer.

Historical review

This historical review is very brief; it only touches on some of the highlights. Many important contributions are not listed. Before 1950 several well-known investigators were carrying out mammography as an everyday clinical examination. Notable among these were Gershon-Cohen of the United States, Willemin of France, and Leborgne of Uruguay.

In the United States, the subject did not receive much acceptance or application prior to Egan's report of work done at the M. D. Anderson Hospital in Houston. Egan reported excellent results with the use of a "new technique" embodying a soft x-ray beam with

low filtration and an extremely high-detail x-ray film. This work incited the United States Public Health Service to enter into training programs and stimulated many radiologists in the United States to begin work in this field of diagnostic radiology.

Investigators were not completely satisfied with the techniques available and a concerted search has always been carried out for a better one. One of the most significant changes concerned the utilization of a molybdenum anode x-ray tube instead of the conventional tungsten anode tube. The molybdenum tube afforded a spectrum of radiation energy which is quite different and resulted in improved contrast on the mammograms. This work was carried out by Gros and is considered a significant advance by many workers in the field.

Another innovative technique concerned xeroradiography. This technique of examining the breast by ionizing radiation was first tried in the early 1950's, but then it was more or less abandoned by the investigators and the Xerox Corporation, which manufactured the equipment.

A further development has been the acceptance by some members of the radiologic community of a combination of a relatively fast x-ray film and screen combination. This technique has the advantage of a relatively low dosage of radiant energy to the breast.

Other innovations concern the application of computer technology to the interpretation of the mammograms, whether on film or xeroradiograph, and some work has been done showing that perhaps this might be feasible. Many techniques of image enhancement have been tried and are now being developed in an attempt to extract more information from the radiographic image. Without question, newer methods of imaging will be tried and perhaps even better and more precise ones will be developed.

This author has been deeply involved in the research, development, and application of xeroradiography as a method of breast cancer detection and that subject will be treated at length.

Xeroradiography

One of the problems in breast cancer diagnosis is the "dense" or dysplastic breast. It is difficult to identify a small cancer in this type of breast by either radiographic means or physical examination. This is the type of breast in which the film mammogram often fails.

Xeroradiography, which is a photoelectric system of recording the radiographic image, has an inherent physical phenomenon called edge enhancement. Because of this phenomenon, small disparities in density, especially if they are sharp discontinuities, have an exaggerated contrast; this is why it is believed that xeroradiography succeeds more frequently in the difficult breast than do the usual film techniques. In addition, there is no doubt that calcifications are imaged better on the xeroradiograph (XR), especially in the dense breast, and this is another area where film, especially if coupled with a molybdenum anode tube, fails.

Xeroradiography is an outgrowth of the science of xerography, or the duplication of graphic material, which was discovered by Chester Carlson in 1938. In 1948 it was found that the xerographic plates would also respond to ionizing radiation and, therefore, the feasibility of applying this method to the recording of radiographic images was demonstrated.

Early attempts to apply this technique to medicine appeared to fail or, at least, were not astoundingly successful. Work began at Hutzel Hospital in 1966 on this science. Preliminary results were encouraging to many of the radiologic community concerned with breast cancer diagnosis and the Xerox Corporation reopened its research and development in this science. There followed a 4 year period of development and machine construction and today there are many xeroradiographic machines functioning throughout the United States and other parts of the world.

Xeroradiographic process

Xeroradiography is a photoelectric imaging system. The most fundamental and unique part is the xeroradiographic plate. It is a sheet of aluminum which measures about 9 by 14 inches. On the surface is coated a thin layer of selenium—an amorphous, enamel-like substance which is a photoconductor.

In brief, a voltage is placed on the selenium surface and the part to be examined is x-rayed. Where x-rays strike the selenium, photoconduction is produced, causing the differential dissipation of the voltage previously placed on the plate in amounts proportional to the absorption of the x-ray.

By means of photoconduction, a latent electrostatic image is created on the plate's surface. This is made visible by conventional xerographic development: a cloud of finely divided, blue plastic particles is brought into proximity to the plate's surface in a dark enclosure. The particles have an electrostatic charge and they form a powder image on the plate's surface. The powder image is then transferred to a sheet of paper, again in the conventional xerographic manner, and that image, permanently fused on the paper, is available to the radiologist for interpretation.

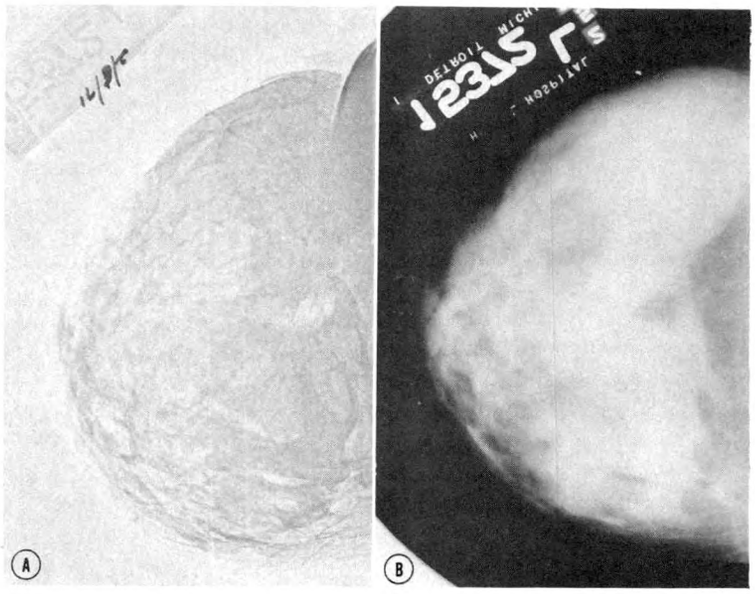


Fig. 1. *(A)* Caudal projection of the breast of a 35-year-old woman involved severely with mammary dysplasia by xeroradiography. *(B)* Caudal projection of the same breast utilizing film mammography. Note how much more detail is visible on the xeroradiograph as compared to the conventional film mammogram.

The images obtained by xeroradiography are believed to have some distinct advantages over film mammography; there is very good resolution. One of the important diagnostic features of breast carcinoma is microcalcifications and there is evidence to support the fact that microcalcifications are more readily identified on the XR image than on the conventional film image. Another advantage of xeroradiography is the wide latitude of structures recorded on one image. That is to say, the nipple and skin and deep structures within the breast are recorded in such a manner that it is easy for the radiologist to extract information. Film does not exhibit this wide latitude.

The edge enhancement enables the XR to "break up" dense structures within the breast into individual components (Fig. 1). This enables small architectural changes, such as spicules around the margin of a mass, to be more readily identified on the XR than on the conventional film. In addition, the xeroradiograph is considered to be highly accurate—more accurate in the hands of this author than conventional film techniques. The technician's work is facilitated in that a darkroom is not required and there is less chance for error in selection of radiographic techniques.

Technique of examination

The technique of examination of the breast radiographically is fairly standard, whether one utilizes xeroradiography or a film system. Physically, the women is examined in two projections at right angles to each other. With conventional radiographic equipment, usually the lateral projection is made with the patient supine, lying on the side to be examined, and the direction of the x-ray beam is mediolateral. A second projection obtained is termed the caudal or cephalocaudal; that is made with the patient sitting, and the direction of the x-ray beam is superoinferior.

Numerous special mammographic machines have been manufactured, each of which is a little different. In some of them the patient stands for the complete examination, in others she sits for the entire examination. Most of these machines, for the lateral projection, will employ an x-ray beam the direction of which is lateromedial. This is considered by some to be disadvantageous in that neither the tail of the breast nor the axilla are routinely imaged and at times some of the deeper structures near the chest wall are not visible on this projection. Not all of the special mammographic machines have this restriction in the way the lateral must be produced. With some the lateral can be produced in the usual mediolateral beam direction, which enables the technologist to image not only the breast but a portion of the chest wall and the axilla. Needless to say, there are advocates of all types of equipment and all types of projections. The choice depends on the radiologist's experience and what he deems to be important to visualize.

The amount of radiation required for mammog-

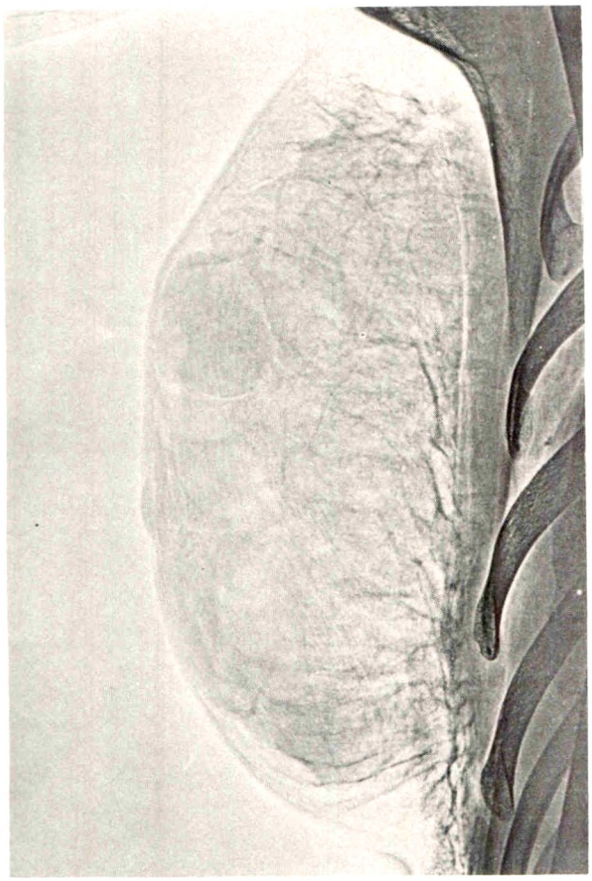


Fig. 2. Lateral projection of the breast of a 27-year-old woman with a palpable mass. The well-defined tumor, bulging the skin located superficially slightly above the level of the nipple, is considered very characteristic of a fibroadenoma.



Fig. 3. Lateral projection of a 62-year-old woman with slight thickening palpable in both breasts. The beaded appearance of the parenchyma is considered very characteristic of a prominent duct pattern.

raphy is modest and it is questioned seriously if the levels utilized in this diagnostic procedure are important as to their carcinogenic effect. To begin, there is an immeasurable dose to the abdomen and neck in our experience. The beam is always tangential to the body except in the occasional axillary view, when it is perpendicular to that area. Dosimetry at the low kVp. level utilized in mammography is difficult to obtain as opposed to dosimetry in general radiography, where higher dose levels are utilized. Therefore, there is not now a lot of confidence in the levels.

Some concept of the dose levels may be gained from the following chart:

	r/exposure
Low kVp. industrial x-ray film (Egan technique)	3-7
Molybdenum anode x-ray tube and medium-speed film	3-12
Molybdenum anode x-ray tube, single intensifying screen, and fast film	<0.75-4
Molybdenum anode x-ray tube and xeroradiography	3-5
Tungsten target x-ray tube and xeroradiography	<0.5-1

Great care must be taken in evaluating radiation dose and consequent information content of the image. In general, with radiographic images, as one diminishes the dose, the information content changes drastically. For example, one could do mammography with very fast screen-film combinations where the dose would be only a small fraction of that usually required. Unfortunately, the information is also but a portion of that obtainable by accepted techniques.

One must also be careful in evaluating data supplied by manufacturers. For example, one manufacturer may state that a system may have a resolution of 10 line pairs per millimeter, which, as a measure of resolution, is considered only acceptable. The important question is: Under what situations does this resolution exist—in

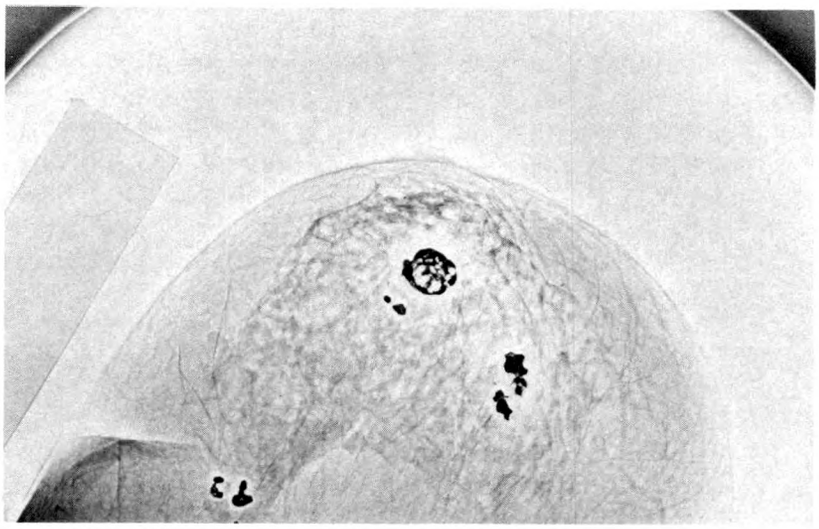


Fig. 4. Caudal projection of a 49-year-old woman with masses palpable in both breasts. The densely calcified masses within the caudal projection of this breast are very characteristic of fibroadenomas. The opposite breast contained four similar tumors.

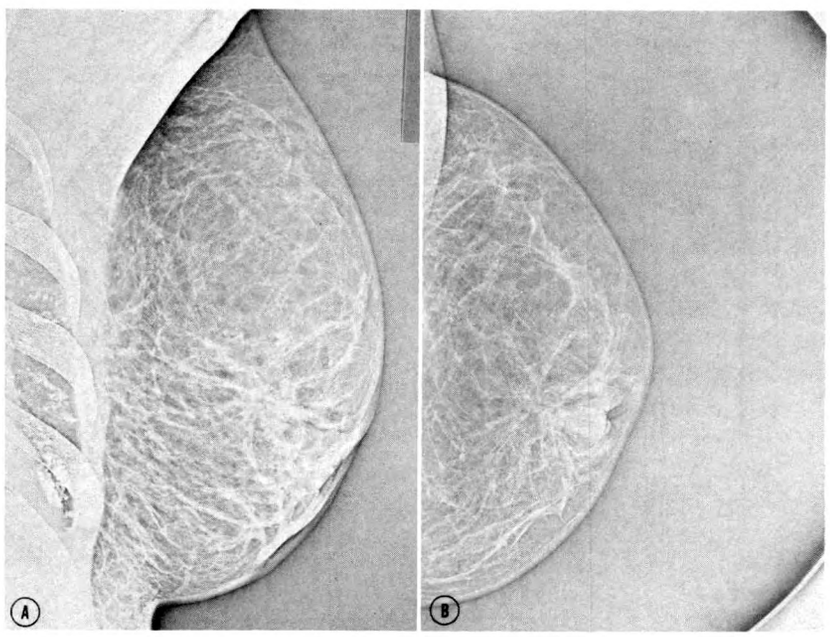


Fig. 5. Both projections of the breast of a 33-year-old woman with a history of chronic breast abscess. She has had an incision and drainage in the subareolar area several times in the past. The deformity of the parenchyma of the breast in the subareolar area is considered characteristic for scarring from a chronic breast abscess.

mammography or some artificial measuring device that bears no relationship to mammography?

The reader is requested to refer to the section on breast screening for a further discussion of dosimetry.

Clinical application

The role of mammography in the symptomatic woman is twofold: to confirm the physical findings and to contribute its own unique information. The physical and mammographic examinations of the breast must be regarded as complementary and should be considered together in decision making but, at the same time, each mode of evaluation must be strongly considered independently. Each mode can, in itself, direct the surgeon toward or away from biopsy. When these methods of examination are not utilized thus,

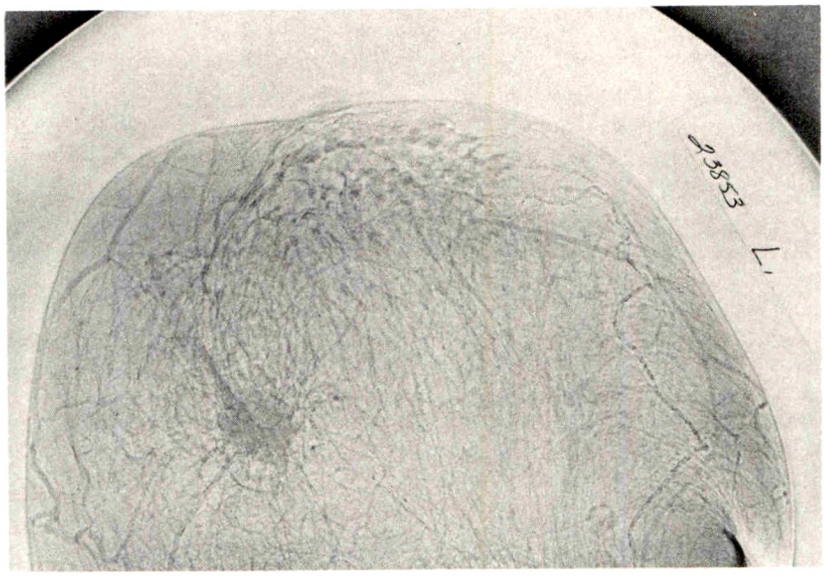


Fig. 6. Caudal projection of the left breast of a 62-year-old woman who had a palpable mass in the medial aspect. The mass with irregular margins is considered very characteristic of a scirrhous carcinoma. In the subareolar area some prominent ducts are also noted.

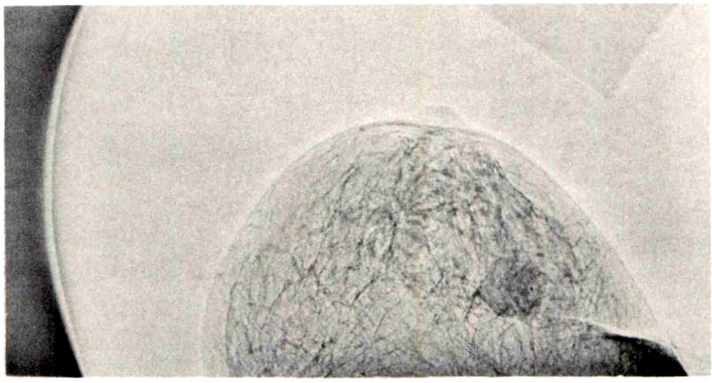


Fig. 7. Caudal projection of the breast of a 46-year-old woman with a palpable mass in the axillary portion. The poorly limited tumor is considered characteristic of a medullary carcinoma.

then certain carcinomas of the breast may suffer from late treatment.

Radiographically, the following divisions of breast conditions can be identified.

1. Normal breast
2. Benign conditions
   a. Mammary dysplasia
   b. Neoplasms
   c. Effects of stasis
   d. Effects of aging
   e. Inflammations
   f. Trauma
   g. Miscellaneous benign conditions
3. Malignancies
   a. Carcinoma
   b. Sarcoma

The normal breast is interesting to the radiologist in that the pathologist rarely tells him it exists and he uncommonly sees images which are regarded as completely normal. The usual patient in whom the radiologist will judge the breasts as normal is the multiparous one, especially if she is near the menopause and slightly obese. Even in that case, there is the risk that the pathologist will describe histologic findings of dysplasia. The very high incidence of connective tissue hyperplasia together with microcyst formation and adenosis, even in the very young girls' breasts (90 to 95 per cent), indicate the developmental nature of these "dysplasias" which one must consider as "normal" (Fig. 2).

Another very interesting observation to be made

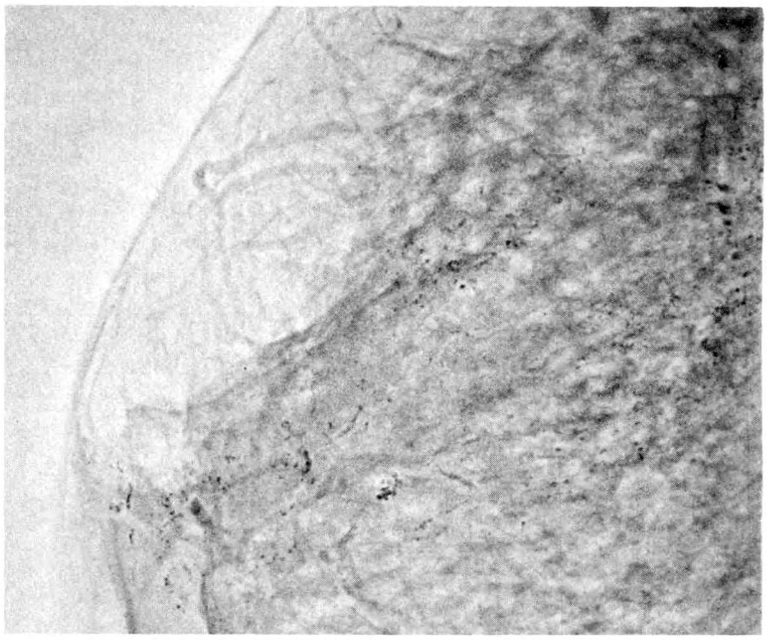


Fig. 8. Photographic enlargement from a portion of the caudal projection of the breast of a 39-year-old woman with no particular palpable abnormality other than slight thickening in both breasts. The calcific deposits are considered very characteristic for a comedocarcinoma. Note that there is widespread involvement and, in spite of the extent of the disease, the tumor was not palpable.

from the radiographic examination of the breast is periductal hyperplasia, or "prominent duct pattern," which has some association with most breast cancers and is also associated most frequently with postmenopausal, nulliparous women. Although cancer of the breast occurs in women without this gross and histologic change, the majority will exhibit radiographically and histologically varying degrees of connective tissue hyperplasia around the circumference of ducts. Indeed, in certain cases, the only radiographic evidence of some breast carcinomas will be an asymmetrical, unilateral prominence of ducts (Fig. 3). The cancer often will be too small to be observed on the mammogram. (See later section on risk factors.)

Many of the benign conditions of the breast have perfectly characteristic radiographic features. A good example is a densely calcified fibroadenoma. In the experience of thousands of these tumors, there has never been a mistake in diagnosis. They have a characteristic lobulated appearance and as myxomatous degeneration occurs they become progressively calcified. These tumors are not confused with carcinoma insofar as their radiographic appearance is concerned (Fig. 4). Some other benign conditions which can produce masses and have rather characteristic radiographic features, especially when coupled with the historical facts of the case, are inflammations of the breast and the effects of trauma (Fig. 5).

The radiographic signs of carcinoma of the breast can be divided into primary and secondary, as shown in the following tabulation.

Primary signs:
- Mass
- Tumor calcifications
- Localized prominent ducts

Secondary signs:
- Thickening of the skin and/or areola
- Retraction of the skin
- Increased vascularity

From the primary signs the radiologist often is capable of being almost 100 per cent confident that a carcinoma exists in the breast when the features are well developed. That is to say, given a mass with a spiculated border and skin retraction, it would be extremely unusual if a finding such as that does not represent a carcinoma (Fig. 6). On the other hand, the radiologist is less confident when encountering a medullary carcinoma (Fig. 7). The differential diagnosis between a medullary carcinoma and a cyst or uncalcified fibroadenoma is exceedingly difficult.

Tumor calcifications when perfectly characteristic can lead to a very firm, positive diagnosis of carcinoma.

Unfortunately, many other benign diseases of the breast also calcify and at times calcify in a manner which may simulate carcinoma. In fact, one of the problems in mammographic diagnosis is diagnosing collections of calcifications, which are in reality associated with benign disease, as being associated with a carcinoma (see Fig. 8).

Regarding secondary signs of carcinoma, skin retraction is exceedingly valuable as a secondary sign but one must be extremely careful that the skin retraction is not deformity of the skin due to previous biopsy. This problem is further complicated by the fact that carcinomas do occur at the site of previous biopsies so at times a radiologist is incapable of coming to a reasonable, accurate decision as to whether or not a particular stellate density is due to previous surgery or a carcinoma.

Another area which can be extremely valuable for identifying some advanced carcinomas is thickening of the skin (see Fig. 9). Here again, however, there are benign conditions which produce skin thickening, especially inflammatory disease. Skin can also be thickened in cases of extensive congestive failure. Areolar thickening should be approached with great caution because low-grade inflammatory diseases of the nipple and areola are exceedingly common and a change of the areolar shadow, such as thickening, is not in itself sufficient for a diagnosis of carcinoma. There is a temptation by some to overread simple areolar thickening as an indication of Paget's carcinoma of the breast. Paget's carcinoma should be accompanied, for a confident radiographic impression, by calcifications in the subareolar area, or somewhere within the breast, indicating the presence of an intraductal carcinoma. Increased vascularity of one breast is the least reliable sign of carcinoma and should be used only to focus greater attention on the breast having the increased vascularity.

A radiologist very often can be firm and decisive in his opinion, but sometimes he cannot. In cases such as that, close cooperation is needed between the radiologist and the referring physician. An example of such cases is illustrated in Fig. 10. The solitary mass, which on physical examination appears smooth and freely movable and which on radiographic examination appears homogeneous, discrete, and circumscribed, is typically a cyst, most likely, or fibroadenoma, with some slight preference being possible on the basis of the patient's age and the general appearance of the breast. These discrete tumors, however, can represent circumscribed forms of carcinoma.

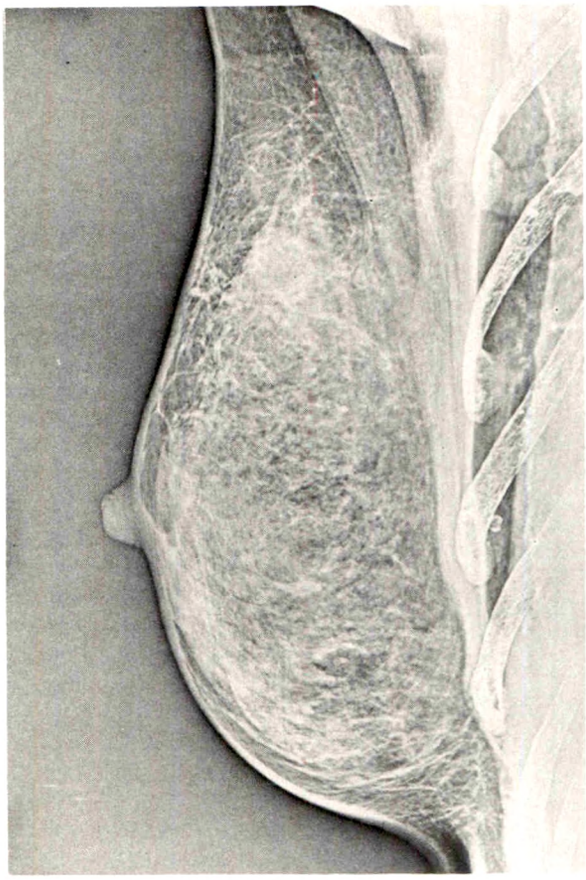


Fig. 9. Lateral projection of the breast of a 52-year-old woman with a palpable mass in the upper portion of the breast. The irregular tumor seen superior to the nipple is considered characteristic of a carcinoma. There is very early involvement of the skin, evidenced by slight thickening in the area of the tumor and, on close inspection, direct continuity between the abnormality of the skin and the tumor could be seen.

These are the sorts of masses that require close cooperation between surgeon and radiologist. Ideally, if the mass is of sufficient size, the surgeon will attempt aspiration of the contents and, if it is cystic and the fluid content is usual, the treatment of that clinical problem is simplified. If the tumor is solid, however, then one must excise it.

There are many instances when the radiologist has to call upon the physical examination for help, either to evaluate a suspected radiographic observation by a directed repeat physical examination or to permit the radiologist to re-examine the patient at short intervals to satisfy himself that an abnormality thought to be benign can be demonstrated to be static in its growth (or regressive) over varying periods of time. This is especially helpful in the case of cysts, as they fluctuate greatly over short periods of time.

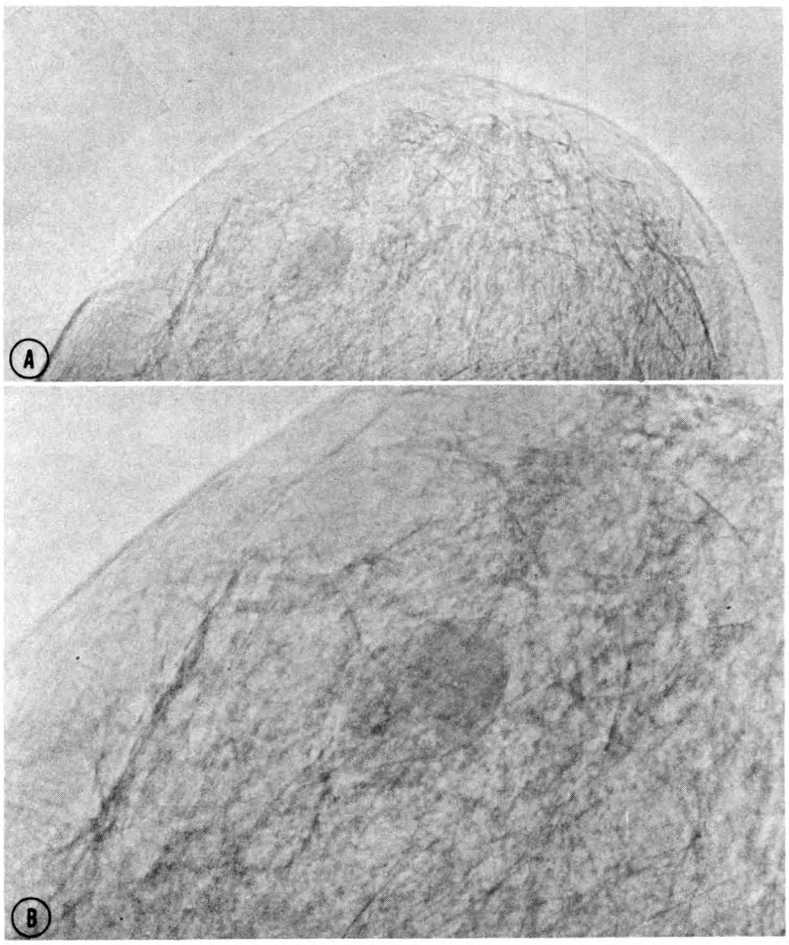


Fig. 10. *(A)* Caudal projection of the breast of a 40-year-old woman with a palpable mass in the axillary quadrant. *(B)* Photographic enlargement of the tumor. This illustrates one of the difficulties in mammography: the inability to differentiate some benign tumors from malignant ones. The tumor was interpreted as representing perhaps a circumscribed carcinoma; it is a fibroadenoma.

Results

The index of success of a mammographic technique is the number of occult carcinomas found and the size of the cancers diagnosed correctly. The over-all accuracy is variable and not a good index of the efficacy of a technique, in that it can be too strongly influenced by the type of case material. Similarly, the presence or absence of metastatic disease in axillary nodes is no longer valid as an index because the type of operative procedure varies so much from institution to institution and the zeal of the pathologist in searching for the nodes varies. It is simple to measure the area of involvement from the mammogram and tumor size relates in a large proportion of cases to stage of disease. One can also make a reasonable judgment as to whether the cancer was occult to physical examination and certainly this is an index of the worth of the procedure.

A total of 462 carcinomas of the breast were observed by xeroradiography at Hutzel Hospital during a 4 year period. These occurred in a referral-type practice which today has an incidence of carcinoma of a little less than 2 per cent of those examined. A true positive rate of 95.3 per cent was attained, while maintaining a false-positive rate of about 5 per cent. The age distribution was significant in that 35 per cent of the patients were less than 50 years of age. The tumor size was even more remarkable in that 31 per cent were judged 1.0 cm. or less.

The most interesting aspect of the analysis concerned the group of carcinomas judged "occult." Typically they were small, 59 per cent of the cases being 1.0 cm. or less in diameter, which is not surprising. Carcinomas in young women tended to be more often occult than those in older women: 30 per cent for the entire group and 43.2 per cent of cancer in

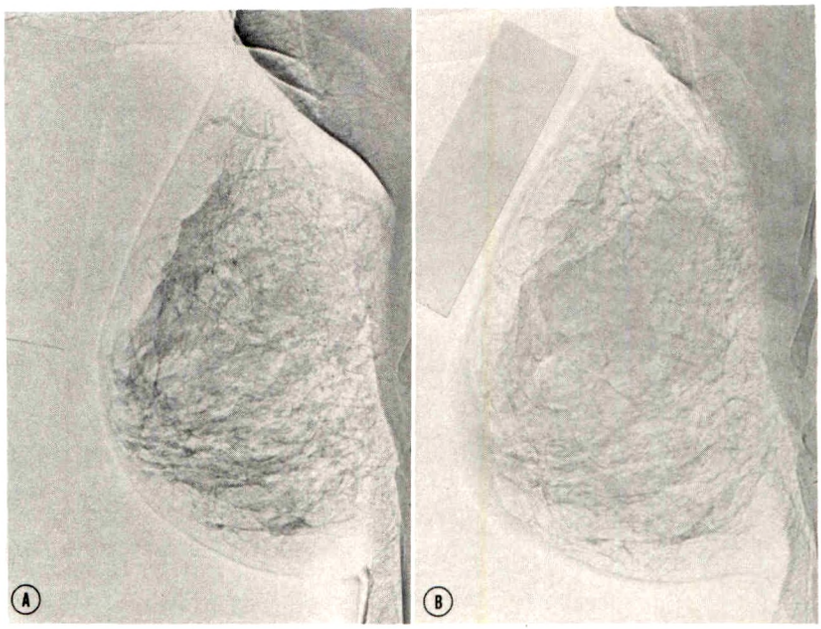


Fig. 11. *(A)* Lateral projection of the breast of a 43-year-old woman who had slight thickening palpable in the upper quadrants of both breasts. The interpretation was benign mammary dysplasia and no evidence of carcinoma. *(B)* The same patient on repeat examination 6 months later. There is now a very large carcinoma occupying an area of approximately 3 by 4 cm. with marked edema of the areola and the skin. At the time of operation, many lymph nodes were palpable.

the age group 31 to 40, vs. 18.7 per cent for women over the age of 71. The high incidence of occult cancer in young women is not too surprising when one considers that their breasts are much more difficult to examine by palpation as compared to the breasts of older, postmenopausal women.

Occult cancers, if they are represented as a mass, are typically small, some of 2 and 3 mm. being observed. These are uncommonly observed, however, in the absence of calcifications characteristic of carcinoma. Some carcinomas attain a size of 6 to 8 cm. and are still nonpalpable. Typically these are comedocarcinomas in dysplastic breasts which are still "early" in their invasive growth and have not evoked a connective tissue response within the neoplastic region.

The type of breast in which the radiologist is most likely to err in his diagnosis is the small, dense one, involved severely with connective hyperplasia either in association with cystic disease and adenosis or periductal connective tissue hyperplasia. In addition, the cancers most often "missed" are probably rapidly growing ones that do not form distinct masses or develop tumor calcifications (Fig. 11).

Breast cancer screening

At the time of this writing there are 27 Breast Cancer Screening Centers in operation throughout the United States operated conjointly by the National Institute of Health and the American Cancer Society. Mammography has demonstrated an effectiveness in discovering small tumors long before they become palpable. It has long been believed by most people that early diagnosis of a breast cancer should lead to an improved cure rate. This seems to have been settled decisively by the study conducted by the Health Insurance Plan of Greater New York, where it was found that, when a population was divided into two groups and half were screened and half were not, the mortality rate from breast cancer was greater in the nonscreened group.

It is likely that, until a better method of finding the small, early breast cancer is discovered, screening by mammography will become more and more popular and should be successful in the limited amount of the population it can be applied to.

Breast cancer screening centers may be aided greatly by the discovery of risk groups for breast cancer, so that the population then can be restricted insofar as annual examinations are concerned. To sum up this work, there is a good chance that this may occur in that two groups of patients have been identified, of which one has an incidence of developing breast carcinoma 22 times greater than the other.

Some fear that repeated screenings of the breast may produce more cancer than those that are found. The

only basis for such feeling is that several groups of patients exposed to high doses of radiation subsequently showed an increased incidence of breast cancer. The first of these were the women who were exposed to radiation during the atomic bombings in Japan; the second, groups of women who had repeated fluoroscopic examinations of the chest for pneumothorax therapy in the treatment of tuberculosis.

One must keep in mind the effect of radiation and its relationship to dose and time. For example, a high dose over a very short period of time is much more effective in terms of radiation therapy or destruction of tissue than the same amount of radiation given over a protracted period of time. This is the corollary one might apply to the relatively high doses received by women who were victims of the bombings and those who received the fluoroscopic examinations. They were high doses over a short period of time. Mammography at most is a low dose to a small volume of tissue over a very long period of time (30 years) rather than instantaneous, as in the case of the bombings, or over a 1 or 2 year period, as in the repeated fluoroscopies. In addition, and equally important, it is likely that present-day techniques in mammography, especially as practiced in the United States, deliver considerably less radiation than the dosages received in mammography as it was practiced 5 or 10 years ago.

Future developments

Mammography has changed a great deal in the last 15 years in the United States. It continues to change as more and more of the x-ray manufacturers are devoting considerable amounts of money to equipment and to new film techniques. In addition, methods of extracting information from the film, as by image enhancement techniques, are being developed. In the film industry a combination of film and intensifying screen is now available for the use of those who find it satisfactory and providing sufficient information for their needs. Xeroradiography is changing in that some newer techniques permit a dosage level reduction by about 60 per cent.

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ITEMS

Fourth Annual Newborn Symposium: Major Dilemmas in Neonatal Pediatrics

On April 20 and 21, 1976, Methodist Hospital, Indianapolis, Indiana, once again will host its annual Newborn Symposium, "Major Dilemmas in Neonatal Pediatrics." Among topics to be discussed are the adoptive neonate, dilemmas of maternal-infant interaction, phototherapy hazards, and long-range follow-up of both the congenitally deformed child and the survivors of neonatal intensive care. Guest faculty will include Dr. T. Berry Brazelton (Harvard), Dr. Murray Feingold (Tufts), Dr. Gerard Odell (Johns Hopkins), Dr. Henry Seidel (Johns Hopkins), and Dr. Philip Sunshine (Stanford).

For further details, please write: Dr. Richard S. Baum, The Newborn Center, Methodist Hospital, 1604 N. Capitol Ave., Indianapolis, Indiana 46202. Telephone: (317) 924-8174.

Recent Advances in Reproductive Immunology

The Department of Obstetrics and Gynecology of the University of Iowa College of Medicine announces a postgraduate course entitled, "Recent Advances in Reproductive Immunology," to be held April 29 and 30, 1976. It will include a review of basic immunologic mechanisms as well as a survey of the latest developments in immunologic aspects of infertility, pregnancy problems, and gynecologic oncology by physicians specializing in each field. The registration fee is $125.00 (residents $75.00). For registration and information, contact: Office of Continuing Medical Education, The University of Iowa College of Medicine, 101 CMAB, Iowa City, Iowa 52242.

Symposium on Women at Work

A symposium on Women at Work will be held at the City of Hope Medical Center in Duarte, California, September 29 and 30 and October 1, 1976. The increasing number of working women and emphasis on equality of opportunity at the work site raise important questions about the risk potential to women in the occupational environment.

The conference will address itself to: (1) the current state of knowledge with respect to anatomic, physiologic, psychologic, sociologic, reproductive, legal, and legislative facets of women in the workplace; (2) important sex differences which must be analyzed; (3) the generation of recommendations for employers, labor groups, and governmental agencies with respect to health-care policies.

Those wishing to present papers should submit an abstract by March 1, 1976, to: Dr. E. R. Plunkett, City of Hope Medical Center, Section on Occupational Health, 1500 E. Duarte Rd., Duarte, California 91010.

National Foundation–March of Dimes Symposium on Inborn Errors of Metabolism

The National Foundation–March of Dimes Symposium on Inborn Errors of Metabolism will be held Wednesday, March 24, 1976, at the William Penn Hotel in Pittsburgh, Pennsylvania. Participants include Barton Childs, M.D., R. J. Desnick, Ph.D., M.D., Prof. Dr. Hans. Galjaard, William J. Mellman, M.D., Elizabeth F. Neufeld, M.D., Leon E. Rosenberg, M.D., and Harvey L. Sharp, M.D. For information or registration forms, please write or call: Mr. Robert McFarland, Greater Pittsburgh Chapter, National Foundation–March of Dimes, 1310 Fulton Bldg., Pittsburgh, Pennsylvania 15222. Telephone: (412) 391-3193.

Fourth Annual Breast-feeding Seminar for Physicians

La Leche League International is sponsoring its Fourth Annual Breast-feeding Seminar for Physicians on March 13, 1976, at the San Francisco Hilton Hotel from 9:00 A.M. to 4:30 P.M. For further information, contact: Carol Kolar, R.N., Coordinator, Continuing Medical Education, La Leche League International, Inc., 9616 Minneapolis Ave., Franklin Park, Illinois 60131. Telephone: (312) 455-7730.

Seminars for health professionals

The Women's Health Care Training Programs and Family Planning Clinic of the Department of Obstetrics and Gynecology, Harbor General Hospital, Torrance, California, is sponsoring a series of twelve seminars for nurse practitioners, registered nurses, physicians, and other health professionals. The seminars will be held January 8, February 19, March 11, April 8, May 13, June 10, July 8, August 12, September 9, October 14, November 11, and December 9, 1976. Fee: $100.00 for

all twelve seminars or $15.00 per seminar. These seminars are approved by the California Medical Association (Category 1) for up to three hours of credit in Continuing Education per seminar. The Course Chairman is J. Robert Bragonier, M.D., Ph.D. For information and registration, write to: Ms. Ellen Woolfson, Continuing Education Coordinator, Professional Staff Association, 1124 W. Carson St., Torrance, California 90502. Telephone: (213) 775-7711, extension 1373.

Gynecologic Endocrinology: A postgraduate education course for the practicing physician

The Department of Obstetrics and Gynecology at the University of Tennessee Center for the Health Sciences, Memphis, Tennessee, will sponsor a three-day postgraduate course for clinicians on "Gynecologic Endocrinology," at the Hilton Inn-Memphis Airport, March 11, 12, and 13, 1976. The diagnosis and management of the major gynecologic endocrine disorders will be discussed by 11 visiting faculty and 10 local faculty. Guest faculty include: Drs. Carl E. Cassidy, Charles Flowers, Joseph W. Goldzieher, Robert B. Greenblatt, Jerome M. Hershman, Ronald K. Kalkhoff, Janet W. McArthur, Robert L. Rosenfield, Pentti K. Siiteri, Leon Speroff, and Michael Y. Yannone. An interesting and informative program is also planned for wives, including a pilgrimage to restored antebellum homes. For furthur information and registration, contact: James R. Givens, M.D., 800 Madison Ave., Memphis, Tennessee 38163.

New nomenclature for vulvar disease

The only purpose for a uniform terminology of any group of diseases is to enable a physician to diagnose a condition utilizing a term which indicates the biological behavior of the disease and characterizes its clinical significance. Such prediction of behavior is based on the documented experience of others and will have validity and reliability in porportion to the uniformity, comparability, and volume of that documentation.

At the present time, the diagnosis of many vulvar diseases is imprecise because of lack of a standard terminology, making the available data confusing and the experience nonadditive.

Therefore, it is essential that an international language be developed and employed in all future reports and, insofar as possible, that all past publications be reviewed and reconsidered in the light of this new schema. Only by so doing can meaningful data be obtained.

Use of the new nomenclature* will permit simple and rapid grouping of cases. If a large enough number of cases in any single group show a unique pattern of biological behavior different from other cases within the same group and identifiable as such—then further subdivision of the group may be warranted.

The following terms have been deleted from the vocabulary of vulvar diseases because of the confusion associated with their use: lichen sclerosus et atrophicus, leukoplakia, neurodermatitis, leukeratosis, Bowen's disease, erythroplasia of Queyrat, carcinoma simplex, leukoplakic vulvitis, hyperplastic vulvitis, and kraurosis vulvae.

To replace these terms, the International Society for the Study of Vulvar Disease adopted the new nomenclature outlined in the following classification. This outline is not intended to be a complete discussion of the various entities but rather defines them with a new and simplified terminology.

Vulvar dystrophies. The term "dystrophy" characterizes the many disorders of epithelial growth and nutrition which result in otherwise unclassified alterations of the surface architecture. Included within the general classification of the vulvar dystrophies are those vulvar changes variously described in the past as leukoplakia, kraurosis, atrophic dystrophy, hyperplastic vulvitis, leukoplakic vulvitis, neurodermatitis, etc. The gross changes seen on the vulva may be diffuse or localized and may be seen as thickened or thinned. White or red color changes may be evident. These lesions should be specifically classified according to their microscopic features as follows:

I. Hyperplastic dystrophy
   a. Without atypia
   b. With atypia
II. Lichen sclerosus
III. Mixed dystrophy (lichen sclerosus with foci of epithelial hyperplasia)
   a. Without atypia
   b. With atypia

Vulvar atypia. Atypia (dysplasia, atypical hyperplasia) is further categorized as mild, moderate, or severe, depending upon the extent of the change and may exist:

a. Without dystrophy
b. With dystrophy

Paget's disease of the vulva. Paget's disease of the vulva should be recognized as a distinct clinicopathologic entity with a pathognomonic histologic appearance. Cases of Paget's disease of the vulva should not be considered together with squamous cell carcinoma in situ, since the biological behavior and significance of these two diseases are distinctly different.

Squamous cell carcinoma in situ. Carcinoma in situ may present clinically at any age as papules or macules, coalescent or discrete, single or multiple. Such lesions

*Proposed and adopted by the International Society for the Study of Vulvar Disease at the Second International Congress, January 10, 1975, Key Biscayne, Florida.

may be hyperpigmented (dark), lichenified and hyperkeratotic (white), eczematoid and scaly (pink), or erythroplastic (red), depending on their location (mucous or cutaneous surface) and prior treatment, as well as local hygiene. These varieties of clinical appearance do not warrant the separate titles and subdivisions applied in the past (Bowen's disease, erythroplasia of Queyrat, carcinoma simplex).

Histopathologic definitions.

Dystrophy.

I. Hyperplastic dystrophy

a. Without atypia—epithelial hyperplasia is present. There is acanthosis with elongation of the epithelial folds which may also exhibit blunting and slight edema. Varying degrees of hyperkeratosis are present. Chronic inflammation within the dermis will vary from mild to severe. No atypia is present.

b. With atypia—mild, moderate, or severe. (See definitions of atypia.)

II. Lichen sclerosus

There are variable degrees of thinning of the squamous epithelium with progressive loss of the epithelial folds (rete ridges). Vacuoles may be noted in the basal layer of cells. The dermis just beneath the squamous epithelium has a characteristic acellular, homogeneous appearance. Edema may be present in this area. Deep to the homogeneous zone, infiltration with lymphocytes and plasma cells may vary from mild to severe. Hyperkeratosis may be present.

III. Mixed dystrophy

a. Without atypia—the mixed dystrophy consists of changes consistent with those of lichen sclerosus but with areas of epithelial hyperplasia as described under "Hyperplastic dystrophy" above.

b. With atypia—mild, moderate, or severe. (See definitions of atypia.)

Atypia.

MILD ATYPIA. There is an excessive proliferation of squamous cells, some of which contain nuclei that are enlarged and hyperchromatic and show fine chromatin clumping. The cytoplasm around the nuclei shows a normal maturation process so that as the surface is approached the nuclei become more widely separated from one another. Individually keratinized cells and mitotic figures above the basal layer are rare.

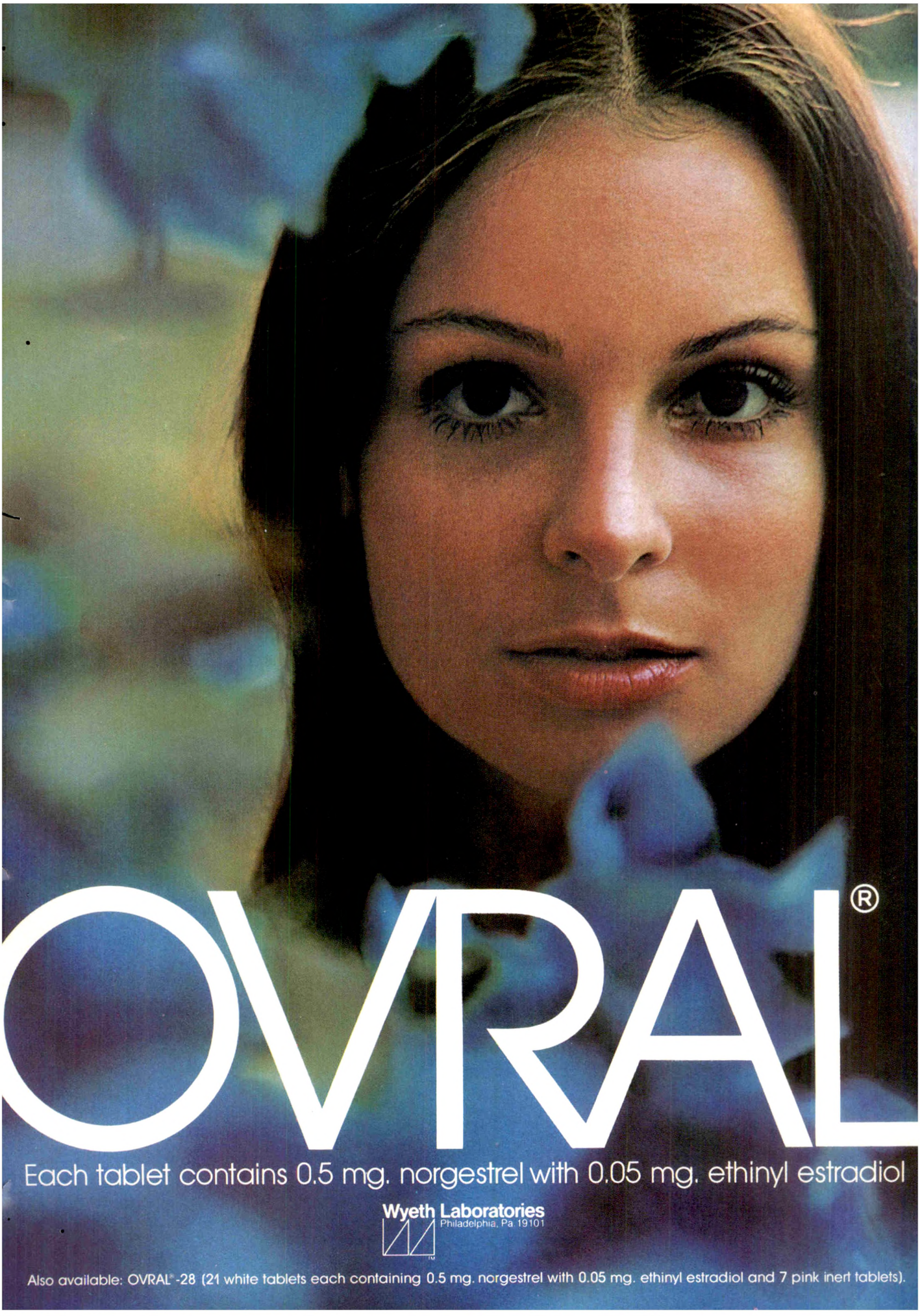
MODERATE ATYPIA. A moderate number of enlarged nuclei are seen which are hyperchromatic and irregular with fine to moderately coarse chromatin clumping. There is partial cytoplasmic maturation of the cells so that as the surface is approached the nuclei show some degree of increased separation from each other due to increased quantities of cytoplasm around each nucleus. As a result of these changes, the appearance is given of increased cellularity and cellular disarray extending through approximately two thirds of the thickness of the epithelium. Individually keratinized cells and mitotic figures above the basal layer are infrequent.

SEVERE ATYPIA. A marked discrepancy in the nuclear/cytoplasmic ratio is present. Nuclei are enlarged and hyperchromatic and show chromatin clumping that may be moderately coarse and irregular. There is little maturation of the cytoplasm so that cells of the parabasal type reach almost to the surface. As they approach the surface, however, some cytoplasmic maturation is seen such that the nuclei appear to be further separated, one from the other, when compared to the deeper layers of the epithelium. The cells in the upper layers are horizontally oriented. Some pyknosis of the surface nuclei may be seen. The appearance is given of cellular atypia extending through more than two thirds of the full thickness of the epithelium with just the upper layers of the epithelium demonstrating cytoplasmic and nuclear maturation. Individually keratinized cells and mitotic figures above the basal layer are seen occasionally.

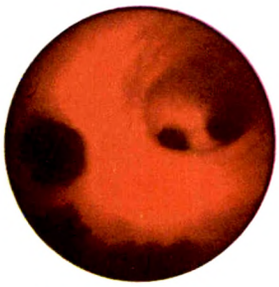
Note: Varying degrees of hyperkeratosis and parakeratosis may be seen in all grades of atypia.

Paget's disease of the vulva. The pathognomonic Paget cells are present. These are large cells which have a clear, vacuolated cytoplasm that can be shown to contain mucopolysaccharides. These characteristic cells are noted initially in the basal layers of the epidermis but later may involve the full thickness of the epithelium as well as the underlying skin adnexa.

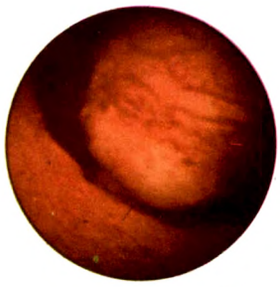
Squamous cell carcinoma in situ. The histologic appearance of squamous cell carcinoma in situ is characterized by disorientation and a loss of epithelial architecture which extends throughout the full thickness of the epithelium (not including the most superficial layers in tissue from keratinized surfaces). Giant cells, multinucleated cells, abnormalities of the nuclear/cytoplasmic ratio, dyskeratosis, individual-cell keratinization, corps rond formation, abnormal mitoses, mitotic figures above the basal layer, and an increased density of cell population may all be seen in varying degrees. Less commonly, full-thickness change is not present but intraepithelial squamous "pearls" are present at the tips of the rete pegs, associated with loss of the normal basal cell layer.


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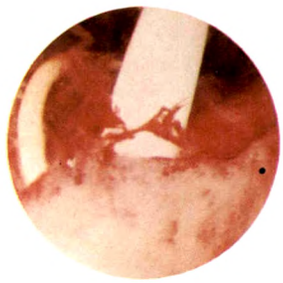
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an endometrial polyp



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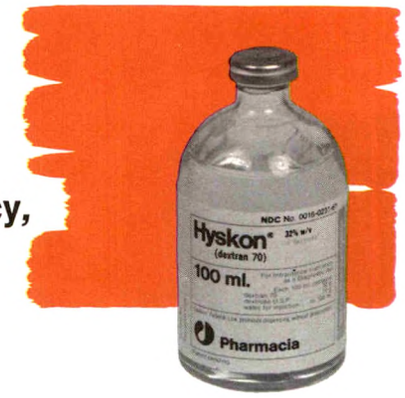
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7. Symposium. Moderator: Levine, R. U. Participants: Siegler, A. M., Quinones, Guerrero, R., Lindemann, H. Contemporary OB/GYN *3* (4):115, 1974.
8. Siegler, A. M.: Medical Tribune, Nov. 7, 1973.

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DESCRIPTION: Hyskon Hysteroscopy Fluid is a clear, viscid, sterile, non-pyrogenic solution of dextran 70 (32% W/V) in dextrose (10% W/V). Dextran 70 is that fraction of dextran, a branched polysaccharide composed of glucose units, having a weight average molecular weight of 70,000. The fluid is electrolyte-free and non-conductive. At room temperature Hyskon Hysteroscopy Fluid has a viscosity of 220 cSt.

Hyskon has a tendency to crystallize when subjected to temperature variations or when stored for long periods. If flakes of dextran are present, heat at 100°-110° C until complete dissolution is achieved.

INDICATIONS: Hyskon Hysteroscopy Fluid is indicated for use with the hysteroscope as an aid in distending the uterine cavity and in irrigating and visualizing its surfaces.

CONTRAINDICATIONS: Hyskon Hysteroscopy Fluid should not be instilled in patients known to be hypersensitive to dextran. All other contraindications are those relative to the hysteroscopic procedure itself, such as pregnancy, endometrial carcinoma, etc.

WARNINGS: It is not known whether systemic absorption of dextran 70 occurs from the uterine and peritoneal cavities. Because of the possibility that absorption can occur, the physician should familiarize himself with the systemic effects of this drug. The most alarming are severe, sometimes fatal anaphylactoid reactions, which are quite rare. Other noteworthy effects include plasma volume expansion and transient prolongation of bleeding time.

ADVERSE REACTION: Undesirable effects directly attributable to the use of Hyskon Hysteroscopy Fluid have not been reported. However, the physician should be mindful of the fact that adverse reactions are known to occur with the intravenous administration of dextran 70 (MACRODEX 6% W/V). Such reactions have included: allergic phenomena (anaphylactoid reactions, urticaria, nasal congestion, wheezing, tightness of chest and mild hypotension), nausea, vomiting, fever and joint pains.

DOSAGE AND ADMINISTRATION: The amount of Hyskon Hysteroscopy Fluid required per patient depends on a number of factors, such as the type and length of the diagnostic procedure, whether or not manipulation or surgery is performed, etc. Most often, however, the amount of Hyskon instilled into the uterus will be between 50 and 100 milliliters.

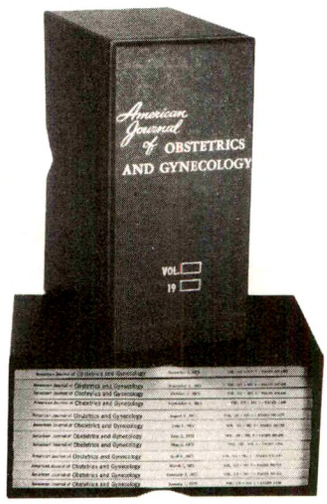
Hyskon should be introduced into the uterine cavity through the cannula of a hysteroscope under low pressure (approximately 100 mm Hg) until the uterus is sufficiently distended to permit adequate visualization. During the hysteroscopic examination, Hyskon should be infused at a rate that keeps the cavity suitably distended. To avoid injection of the fluid into the tissues of the uterus and parametria and to avoid having unnecessary amounts of the fluid pass into the peritoneal cavity and backwards along the side of the hysteroscope, infusion pressures greater than 150 mm Hg should not be used.

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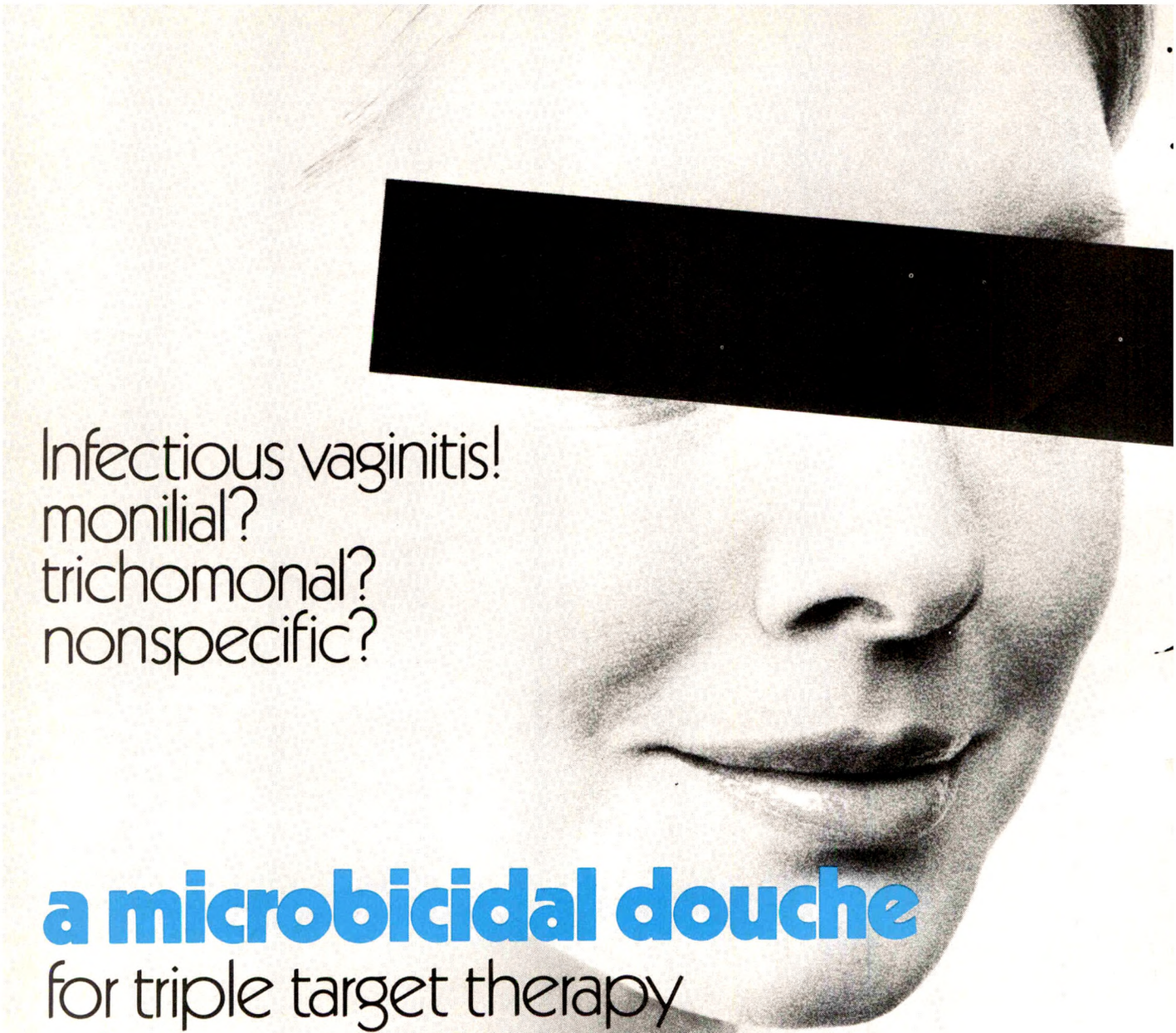
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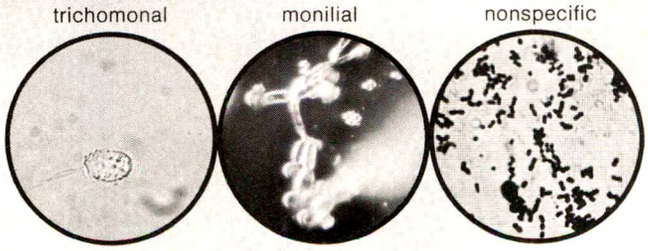
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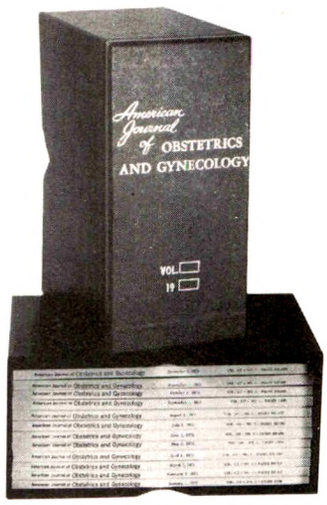
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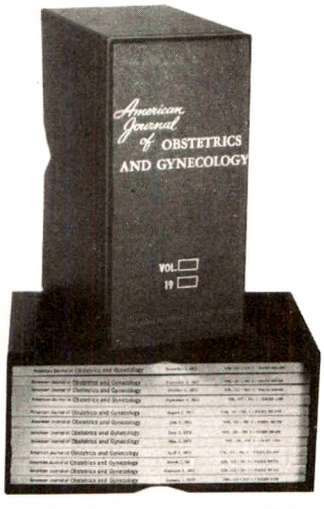
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Official languages will be English and French with simultaneous translation available.

Registration will be closed April 1st 1976. The regular fee will be: physicians, one hundred and fifty canadian dollars ($150.00); nurses, fifty dollars ($50.00), including three breakfasts and two lunches at the Château Frontenac. For registration after April 1st, the fee will be: physicians, two hundred dollars ($200.00); nurses, seventy-five dollars ($75.00). Cheques should be made payable to: Symposium de monitorage foetal, Département d'Obstétrique-Gynécologie, Faculté de Médecine, Cité Universitaire, Québec, Canada, G1K 7P4.

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item:	**Only 35% of all women polled reported that a *physician* had ever raised the subject of breast self-examination, and only 24% had received instruction from the physician on how to do it. Even among women who regularly see a gynecologist, only 34% had been instructed on BSE.**
item:	***But*, among women who received personal instruction from their physicians, the overwhelming majority *(92%)* practiced BSE during the preceding year.**

The Gallup study revealed that, far more important than increasing awareness of breast self-examination, is the problem of inducing women to practice it regularly. The physician plays a key role in this—by teaching women the correct technique, and instilling in them the confidence that will assure their continued practice of BSE.

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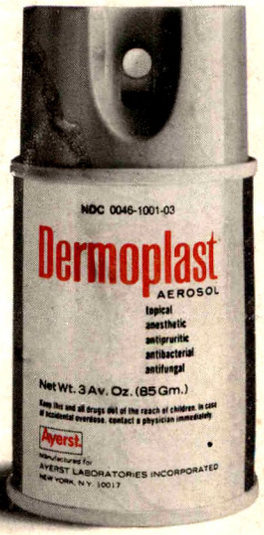
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Special note—Oral contraceptives have been marketed in the United States since 1960. Reported pregnancy rates vary from product to product. The effectiveness of the sequential products appears to be somewhat lower than that of the combination products. Both types provide almost completely effective contraception.

An increased risk of thrombotic, thromboembolic and cerebral vascular disease associated with the use of hormonal contraceptives has been shown in studies conducted in both Great Britain and the United States. British studies have shown an increased risk of myocardial infarction. Other risks, such as those of elevated blood pressure and reduced tolerance to carbohydrates, have been reported and appropriate tests should be conducted to monitor these during oral contraceptive therapy. Liver disease has also been reported, and the physician should be alert to its earliest manifestations.

Long-term administration of both natural and synthetic estrogens in subprimate animal species in multiples of the human dose increases the frequency of some animal carcinomas. These data cannot be transposed directly to man. The possible carcinogenicity due to the estrogens can be neither affirmed nor refuted at this time. Close clinical surveillance of all women taking oral contraceptives must be continued.

Indication—Demulen is indicated for oral contraception.

Contraindications—Patients with thrombophlebitis, thromboembolic disorders, cerebral vascular disease, myocardial infarction or coronary thrombosis, or a past history of these conditions, markedly impaired liver function, known or suspected carcinoma of the breast, known or suspected estrogen-dependent neoplasia, undiagnosed abnormal genital bleeding and known or suspected pregnancy.

Warnings—The physician should be alert to the earliest manifestations of thrombotic and thromboembolic disorders, thrombophlebitis, cerebrovascular disorders including hemorrhage, pulmonary embolism, retinal thrombosis, myocardial infarction or coronary thrombosis. Should any of these occur or be suspected, the drug should be discontinued immediately.

Retrospective studies of morbidity and mortality in Great Britain and studies of morbidity in the United States have shown a statistically significant association between thrombophlebitis, pulmonary embolism and cerebral vascular disease, occlusive or hemorrhagic, and the use of oral contraceptives. There have been three principal studies in Great Britain[1-3] leading to these conclusions and three[4-7] in this country. The estimate of the relative risk of thromboembolism in the study by Vessey and Doll[3] was about sevenfold, while studies in the United States found relative risks of 4.4 to 11, meaning that the users are several times as likely to undergo thromboembolic disease without evident cause as nonusers.

In one British study[8] it was estimated that users of oral contraceptives are 3 to 6 times more likely than nonusers to develop nonfatal myocardial infarction. In another British study[9] it was estimated that users of oral contraceptives between the ages of 40 and 44 were 4 to 5 times more likely to have fatal myocardial infarction than nonusers. Below the age of 40 the relative risk was estimated to be 2 to 3 times as high for users as for nonusers of oral contraceptives. The annual estimated aggregate rate of fatal plus nonfatal myocardial infarction in the 30- to 39-year age group is 4 per 100,000 in nonusers and 11 per 100,000 in users. In the 40- to 44-year age group the aggregate rates are about 22 per 100,000 and 112 per 100,000, respectively. (These estimates are based on British vital statistics, which show acute myocardial infarction death rates 2 to 3 times less than in the U.S. for women in these age groups.) The reports indicate that women with additional risk factors (hypertension, hypercholesterolemia, diabetes, cigarette-smoking, etc.) have a greater risk than the average.

In May 1974 the Royal College of General Practitioners in England[10] issued an interim report of its continuing large-scale prospective study comparing a user group with a nonuser group. This study in its interim analysis states, "A statistically significant higher rate of reporting of cerebrovascular accidents in Takers is evident, but the numbers are too small to justify an estimation of the degree of risk." The study also reported a higher incidence of superficial and deep vein thrombosis in users as compared to nonusers. The Sartwell study[4] indicated that the risk did not persist after discontinuation of administration. Both the Sartwell and the Royal College studies indicated that the degree of risk was not associated with duration of treatment. In a collaborative American study[5,6] of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the relative risk of thrombotic stroke was 4.1 to 9.5 times greater in users than in nonusers. A comparable estimate for hemorrhagic stroke was 2.0. None of the American studies was designed to evaluate a difference between products. However, the Sartwell study suggested that there might be an increased risk of thromboembolic disease in users of sequential products. Other retrospective studies[11,12] have reported an increased risk of postsurgery thromboembolic complications in oral contraceptive users. It has been recommended that therapy be discontinued at least one month prior to elective surgery.

Discontinue medication if there is gradual or sudden partial or complete loss of vision; proptosis or diplopia; onset or aggravation of migraine or development of headache of a new pattern which is recurrent, persistent or severe; papilledema; or any evidence of retinal vascular lesions.

Fetal abnormalities have been reported to occur in the offspring of women who have taken progestogens and/or estrogens during pregnancy.[13-15] The safety of Demulen in pregnancy has not been demonstrated. Pregnancy should be ruled out before initiating or continuing the contraceptive regimen. Pregnancy should always be considered if withdrawal bleeding does not occur.

A small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The long-range effect on the nursing infant cannot be determined at this time.

Hepatic lesions (adenomas, hepatomas, hamartomas, regenerating nodules, etc.), occasionally fatal, have been reported in women on oral contraceptives. Such lesions may present as an abdominal mass or with the signs and symptoms of an acute abdomen. These lesions should be considered if the patient has abdominal pain or evidence of intra-abdominal bleeding. This has been reported in short-term as well as long-term users of oral contraceptives.

Precautions—A thorough history and physical examination should be performed before prescribing oral contraceptives and periodically during their administration and should include special reference to breasts and pelvic organs, including Papanicolaou smears since estrogens have been known to produce tumors, some of them malignant, in five species of subprimate animals. Endocrine and liver function tests may be affected by treatment with Demulen. Therefore, if such tests are abnormal in a patient taking Demulen, it is recommended that they be repeated after the drug has been withdrawn for two months. Under the influence of progestogen-estrogen preparations, preexisting uterine fibromyomas may increase in size. Because these agents may cause some degree of fluid retention, conditions which might be influenced by this factor, such as epilepsy, migraine, asthma, cardiac or renal dysfunction, require careful observation. In breakthrough bleeding, and in all cases of irregular bleeding per vaginam, nonfunctional causes should be borne in mind. In undiagnosed bleeding per vaginam, adequate diagnostic measures are indicated. Patients with a history of psychic depression should be carefully observed and the drug discontinued if the depression recurs to a serious degree. Any possible influence of prolonged Demulen therapy on pituitary, ovarian, adrenal, hepatic or uterine function awaits further study. A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. The mechanism of this decrease is obscure. For this reason, diabetic patients should be carefully observed while receiving Demulen therapy. The age of the patient constitutes no absolute limiting factor, although treatment with Demulen may mask the onset of the climacteric. The pathologist should be advised of Demulen therapy when relevant specimens are submitted. Susceptible women may experience an increase in blood pressure following administration of contraceptive steroids. Cholestatic jaundice has been reported in users of oral contraceptives. If this occurs Demulen should be discontinued. This condition is more likely to occur in patients who have experienced cholestatic jaundice of pregnancy. Patients with a history of cholestatic jaundice of pregnancy should be carefully observed during Demulen therapy.

Adverse reactions observed in patients receiving oral contraceptives—A statistically significant association has been demonstrated between use of oral contraceptives and the following serious adverse reactions: thrombophlebitis, pulmonary embolism, cerebral thrombosis and hemorrhage, myocardial infarction and coronary thrombosis, and gallbladder disease.

Although available evidence is suggestive of an association, such a relationship has been neither confirmed nor refuted for the following serious adverse reactions: neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis, and hepatic lesions with or without intra-abdominal bleeding.

The following adverse reactions are known to occur in patients receiving oral contraceptives: nausea, vomiting, gastrointestinal symptoms (such as abdominal cramps and bloating), breakthrough bleeding, spotting, change in menstrual flow, amenorrhea during and after treatment, edema, chloasma or melasma, which may persist, breast changes (tenderness, enlargement and secretion), change in weight (increase or decrease), changes in cervical erosion and cervical secretions, suppression of lactation when given immediately post partum, cholestatic jaundice, migraine, rash (allergic), rise in blood pressure in susceptible individuals, and mental depression.

Although the following adverse reactions have been reported in users of oral contraceptives, an association has been neither confirmed nor refuted: anovulation post treatment, which tends to occur more frequently in women with a history of menstrual disorders, premenstrual-like syndrome, changes in libido, changes in appetite, cystitis-like syndrome, headache, intolerance to contact lenses, nervousness, dizziness, fatigue, backache, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, itching and vaginitis.

The following laboratory results may be altered by the use of oral contraceptives: hepatic function: increased sulfobromophthalein retention and other tests; coagulation tests: increase in prothrombin, Factors VII, VIII, IX, and X; decrease in anti-thrombin III; increase in platelet aggregability; thyroid function: increase in PBI and butanol extractable protein bound iodine, and decrease in T_3 uptake values; metyrapone test and pregnanediol determination.

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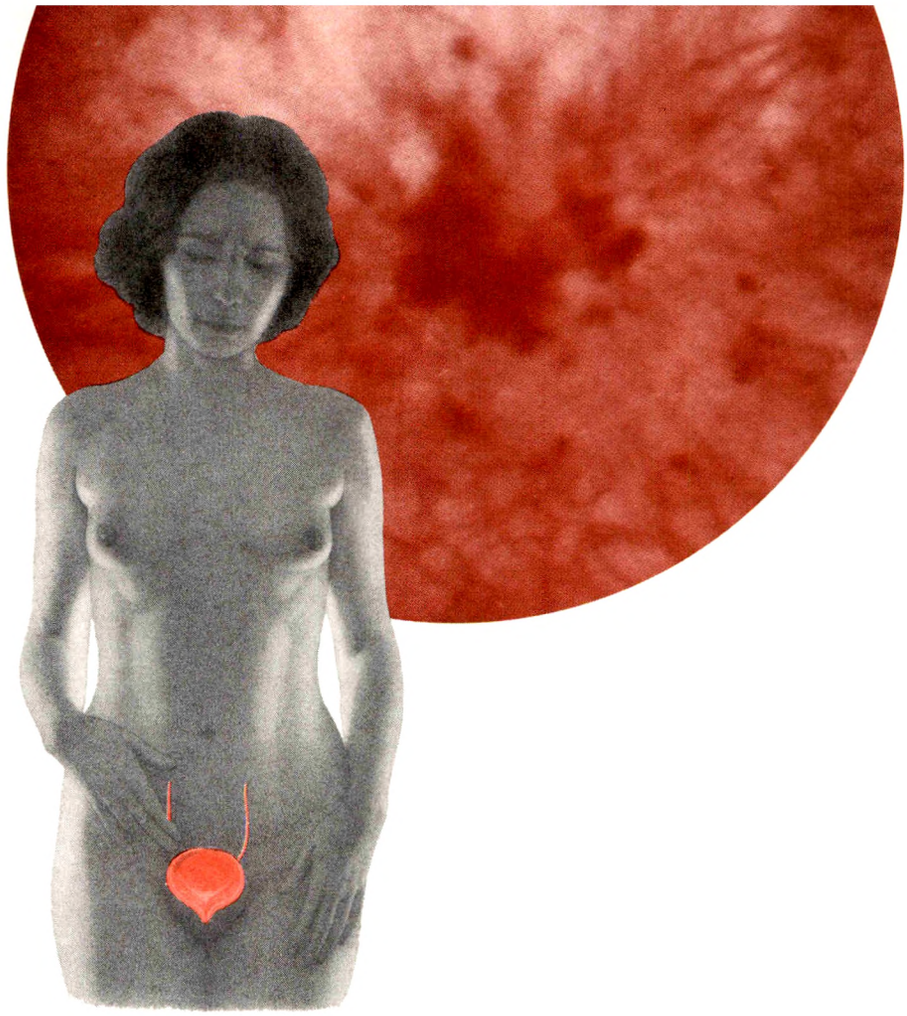
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Important Note: Carefully coordinate *in vitro* sulfonamide sensitivity tests with bacteriologic and clinical response. Add aminobenzoic acid to culture media for patients already taking sulfonamides. Increasing frequency of resistant organisms currently is a limitation of the usefulness of antibacterial agents including the sulfonamides. Blood levels should be measured in patients receiving sulfonamides for serious infections, since there may be wide variations with identical doses; 12 to 15 mg/100 ml is considered optimal for serious infections; 20 mg/100 ml should be the maximum total sulfonamide level, as adverse reactions occur more frequently above this level.

Contraindications: Children below age 12; sulfonamide hypersensitivity; pregnancy at term and during nursing period. Contraindicated in glomerulonephritis, severe hepatitis, uremia, and pyelonephritis of pregnancy with gastrointestinal disturbances, because of phenazopyridine HCl component.

Warnings: Safe use in pregnancy has not been established. Teratogenicity potential has not been thoroughly investigated. Deaths from hypersensitivity reactions, agranulocytosis, aplastic anemia and other blood dyscrasias have been reported; clinical signs such as sore throat, fever, pallor, purpura or jaundice may be early indications of serious blood disorders. Complete blood counts and urinalysis with careful microscopic examination should be performed frequently during sulfonamide therapy.

Precautions: Use with caution in patients with impaired renal or hepatic function, severe allergy, bronchial asthma and in glucose-6-phosphate dehydrogenase-deficient individuals. In the latter, hemolysis may occur. Maintain adequate fluid intake to prevent crystalluria and stone formation.

Adverse Reactions: *Blood dyscrasias:* Agranulocytosis, aplastic anemia, thrombocytopenia, leukopenia, hemolytic anemia, purpura, hypoprothrombinemia and methemoglobinemia. *Allergic reactions:* Erythema multiforme (Stevens-Johnson syndrome), skin eruptions, epidermal necrolysis, urticaria, serum sickness, pruritus, exfoliative dermatitis, anaphylactoid reactions, periorbital edema, conjunctival and scleral injection, photosensitization, arthralgia and allergic myocarditis. *Gastrointestinal reactions:* Nausea, emesis, abdominal pains, hepatitis, diarrhea, anorexia, pancreatitis and stomatitis. *C.N.S. reactions:* Headache, peripheral neuritis, mental depression, convulsions, ataxia, hallucinations, tinnitus, vertigo and insomnia. *Miscellaneous reactions:* Drug fever, chills, toxic nephrosis with oliguria and anuria, polyarteritis nodosa and L.E. phenomenon. Due to certain chemical similarities with some goitrogens, diuretics (acetazolamide and thiazides) and oral hypoglycemic agents, sulfonamides have caused rare instances of goiter production, diuresis and hypoglycemia. Cross-sensitivity with these agents may exist.

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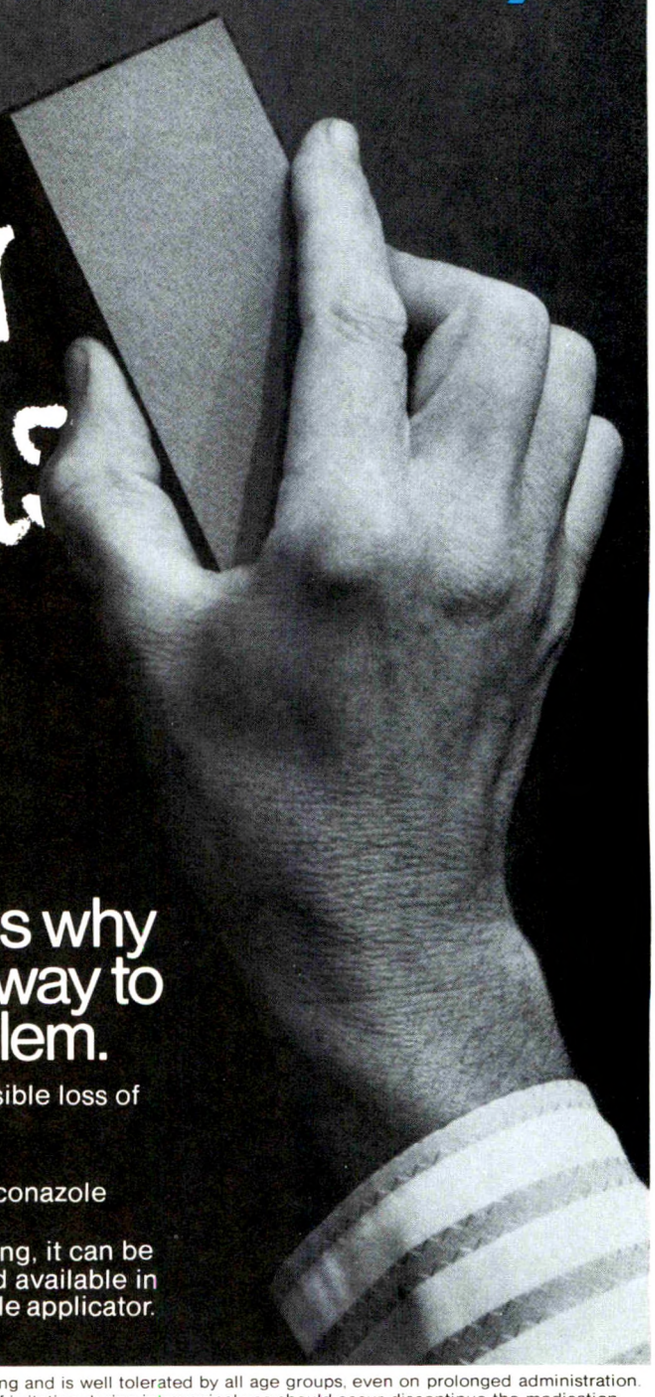
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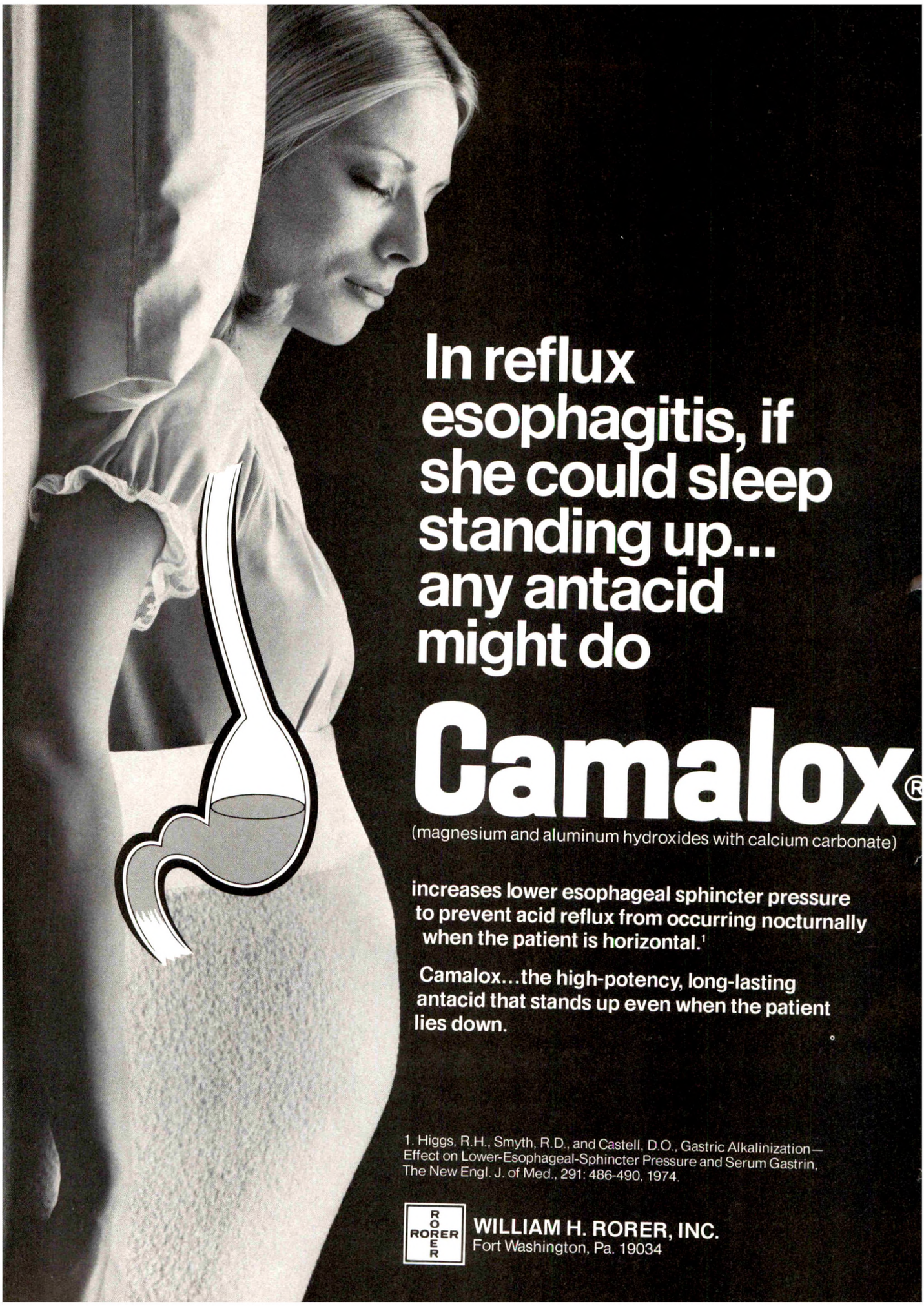
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The causes of vaginitis are multiple



You can depend on AVC... therapy that combats major vaginal pathogens and provides rapid symptomatic relief.*

***Indications:** Based on a review of AVC by the National Academy of Sciences–National Research Council and/or other information, FDA has classified the indications as follows:

"Probably" effective: For the relief of symptoms of vulvovaginitis where isolation of the specific organism responsible (usually *Trichomonas vaginalis, Candida albicans,* or *Hemophilus vaginalis)* is not possible.

NOTE: When the offending organism is known, treatment with a specific agent known to be active against that microorganism is preferred.

"Possibly" effective: For the treatment of trichomoniasis, vulvovaginal candidiasis, and vaginitis due to *Hemophilus vaginalis* or other susceptible bacteria.

Final classification of the less-than-effective indications requires further investigation.

Contraindications: Known sensitivity to sulfonamides.

Precautions/Adverse Reactions: The usual precautions for topical and systemic sulfonamides should be observed because of the possibility of absorption. Burning, increased local discomfort, skin rash or other manifestations of sulfonamide toxicity are reasons to discontinue treatment.

Dosage: 1 applicatorful (about 6 g.) or 1 suppository intravaginally once or twice daily.

Supplied: Cream–4 ounce tube with applicator. Suppositories–Box of 16 with inserter.

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Now, for both aspects of constipation



sluggish bowel

and hard dry stools

Announcing

Senokot® S

(standardized senna concentrate and dioctyl sodium sulfosuccinate) **Tablets**

a unique natural laxative plus a classic stool softener

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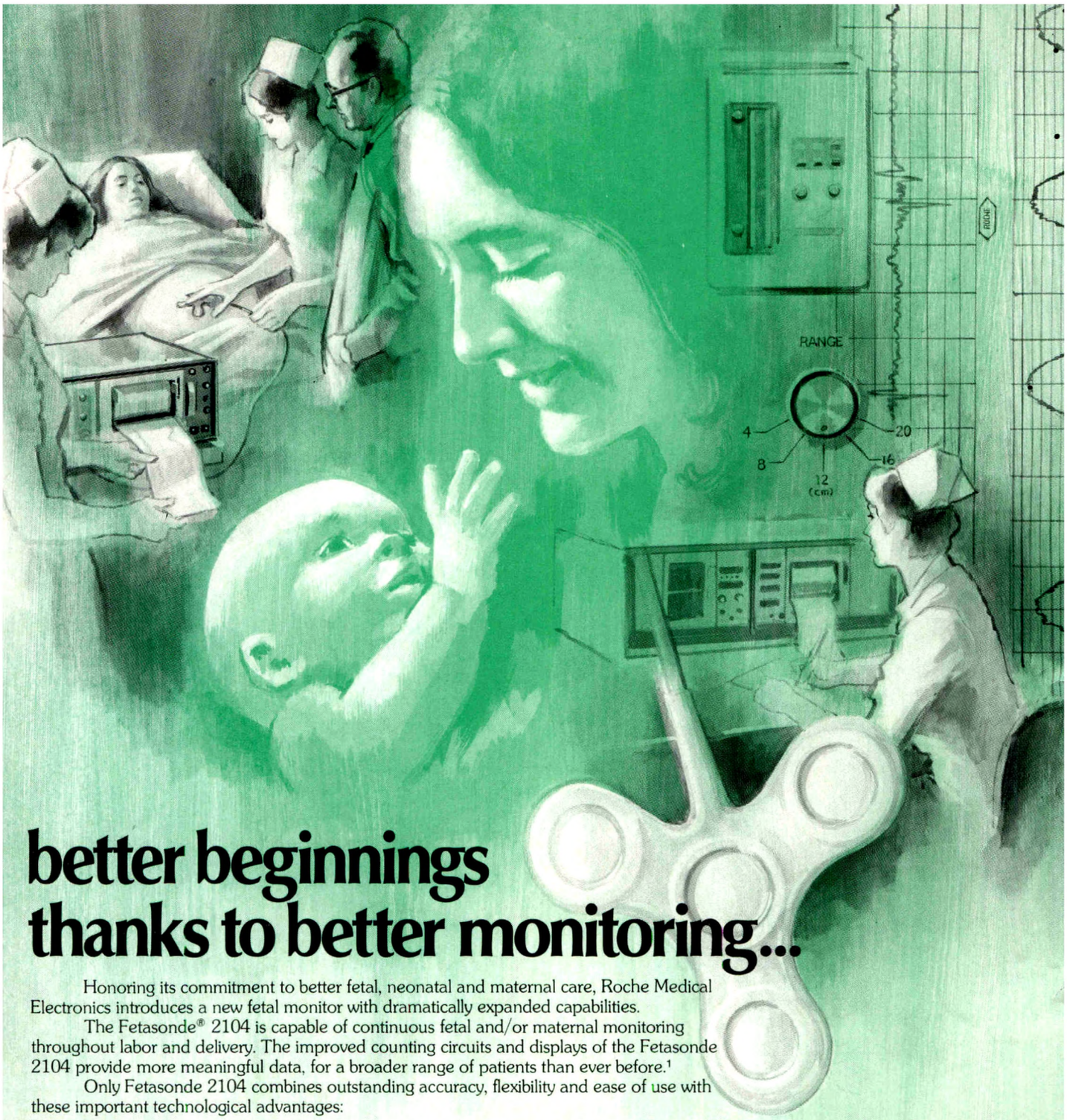
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1. Young, B.K. et al, in press Obstetrics & Gynecology.

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In serious respiratory tract infections...*

*due to indicated susceptible organisms



Until th[illegible] start with 'ANCEF'

Serious respiratory tract infections usually require immediate treatment. In these cases, prudent medical judgment and the use of 'Ancef', when appropriate, can save valuable time while waiting for lab results. 'Ancef' offers:

• Broad coverage of major respiratory pathogens

Streptococcus pneumoniae (formerly *D. pneumoniae*), *Klebsiella* species, *Hemophilus influenzae*, *Staphylococcus aureus* (penicillin-sensitive and penicillin-resistant), and group A beta-hemolytic streptococci.

• High and prolonged serum levels

Single 1 gram IV injections in healthy adult volunteers produced average peak serum levels of 190.0 mcg/ml. At four hours, levels of 16.5 mcg/ml were still present.

• Convenient IV or IM administration at same dosage schedule

When lab tests return, if indicated organism is sensitive to 'Ancef'—and chances are it will be—therapy is already under way. If it isn't sensitive, then 'Ancef' should be discontinued and an appropriate antibiotic should be selected.

Injectable benzathine penicillin is considered the drug of choice in treatment and prevention of streptococcal infections, including prophylaxis of rheumatic fever.

Adverse Reactions

Reported adverse reactions include pain on injection, rash, G.I. symptoms, pruritus and phlebitis. For a complete list of adverse reactions, please see next page.

Warning: In penicillin-sensitive patients, cephalosporins should be used cautiously because of evidence of cross-allergenicity of the penicillins and cephalosporins.

Please see next page for a brief summary of the prescribing information.

SK&F **Smith Kline & French Laboratories**
Division of SmithKline Corporation, Philadelphia, Pa.

ANCEF® IV IM

brand of sterile

CEFAZOLIN SODIUM

(LYOPHILIZED)

Injection: 250 mg, 500 mg, and 1 gram vials

ANCEF® IV IM

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CEFAZOLIN SODIUM

(LYOPHILIZED)

Injection: 250 mg, 500 mg, and 1 gram vials

Before prescribing, see complete prescribing information in SK&F literature or *PDR*. The following is a brief summary.

DESCRIPTION

Ancef® (sterile cefazolin sodium, SK&F) is a semi-synthetic cephalosporin for parenteral administration. It is the sodium salt of 3-{[(5-methyl-1,3,4-thiadiazol-2-yl)thio]methyl}-8-oxo-7-[2-(1H-tetrazol-1-yl) acetamido]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid. The sodium content is 46 mg. per gram of cefazolin.

ACTIONS

Microbiology: *In vitro* tests demonstrate that the bactericidal action of cephalosporins results from inhibition of cell wall synthesis. 'Ancef' is active against the following organisms *in vitro*:

Staphylococcus aureus (penicillin-sensitive and penicillin-resistant)

Group A beta-hemolytic streptococci and other strains of streptococci (many strains of enterococci are resistant).

Streptococcus pneumoniae (formerly *D. pneumoniae*)
Escherichia coli
Proteus mirabilis
Klebsiella species
Enterobacter aerogenes
Hemophilus influenzae

Most strains of *Enterobacter cloacae* and indole positive Proteus (*P. vulgaris, P. morgani, P. rettgeri*) are resistant. Methicillin-resistant staphylococci, serratia, pseudomonas, mima, herellea species are almost uniformly resistant to cefazolin.

INDICATIONS

Ancef (sterile cefazolin sodium, SK&F) is indicated in the treatment of the following serious infections due to susceptible organisms:

Respiratory tract infections due to *Streptococcus pneumoniae* (formerly *D. pneumoniae*), *Klebsiella* species, *Hemophilus influenzae*, *Staphylococcus aureus* (penicillin-sensitive and penicillin-resistant), and group A beta-hemolytic streptococci.

Injectable benzathine penicillin is considered to be the drug of choice in treatment and prevention of streptococcal infections, including the prophylaxis of rheumatic fever. 'Ancef' is effective in the eradication of streptococci from the nasopharynx; however, data establishing the efficacy of 'Ancef' in the subsequent prevention of rheumatic fever are not available at present.

Genitourinary tract infections due to *Escherichia coli, Proteus mirabilis, Klebsiella* species, and some strains of enterobacter and enterococci.

Skin and soft-tissue infections due to *Staphylococcus aureus* (penicillin-sensitive and penicillin-resistant) and group A beta-hemolytic streptococci and other strains of streptococci.

Bone and joint infections due to *Staphylococcus aureus*.

Septicemia due to *Streptococcus pneumoniae* (formerly *D. pneumoniae*), *Staphylococcus aureus* (penicillin-sensitive and penicillin-resistant), *Proteus mirabilis, Escherichia coli*, and *Klebsiella* species.

Endocarditis due to *Staphylococcus aureus* (penicillin-sensitive and penicillin-resistant), and group A beta-hemolytic streptococci.

Appropriate culture and susceptibility studies should be performed to determine susceptibility of the causative organism to 'Ancef'.

CONTRAINDICATIONS

Ancef (sterile cefazolin sodium, SK&F) is contraindicated in patients with known allergy to the cephalosporin group of antibiotics.

WARNINGS

BEFORE CEFAZOLIN THERAPY IS INSTITUTED, CAREFUL INQUIRY SHOULD BE MADE CONCERNING PREVIOUS HYPERSENSITIVITY REACTIONS TO CEPHALOSPORINS AND PENICILLIN. CEPHALOSPORIN C DERIVATIVES SHOULD BE GIVEN CAUTIOUSLY IN PENICILLIN-SENSITIVE PATIENTS.

SERIOUS ACUTE HYPERSENSITIVITY REACTIONS MAY REQUIRE EPINEPHRINE AND OTHER EMERGENCY MEASURES.

There is some clinical and laboratory evidence of partial cross-allergenicity of the penicillins and the cephalosporins. Patients have been reported to have had severe reactions (including anaphylaxis) to both drugs.

Any patient who has demonstrated some form of allergy, particularly to drugs, should receive antibiotics cautiously. No exception should be made with regard to 'Ancef'.

Usage in Pregnancy: Safety of this product for use during pregnancy has not been established.

Usage in Infants: Safety for use in prematures and infants under 1 month of age has not been established.

PRECAUTIONS

Prolonged use of cefazolin sodium may result in the overgrowth of nonsusceptible organisms. Careful clinical observation of the patient is essential.

When 'Ancef' is administered to patients with low urinary output because of impaired renal function, lower daily dosage is required (see dosage instructions). A false positive reaction for glucose in the urine of patients on 'Ancef' has occurred with Clinitest® tablets solution.

ADVERSE REACTIONS

The following reactions have been reported.

Hypersensitivity: Drug fever, skin rash, vulvar pruritus, and eosinophilia have occurred.

Blood: Neutropenia, leukopenia, thrombocythemia and positive direct and indirect Coombs tests have occurred.

Hepatic and Renal: Transient rise in SGOT, SGPT, BUN and alkaline phosphatase levels has been observed without clinical evidence of renal or hepatic impairment.

Gastrointestinal: Nausea, anorexia, vomiting, diarrhea, oral candidiasis (oral thrush) have been reported.

Other: Pain at site of injection after intramuscular administration has occurred, some with induration. Phlebitis at site of injection has been noted.

DOSAGE AND ADMINISTRATION

'Ancef' may be administered intramuscularly or intravenously after reconstitution.

Dosage

In adults, usual dosage for pneumococcal pneumonia is 500 mg. every 12 hours. In acute, uncomplicated urinary tract infections, usual dosage is 1 gram every 12 hours. In mild infections caused by susceptible Gram positive cocci, usual dosage is 250 to 500 mg. every 8 hours. In moderate to severe infections, usual dosage is 500 mg. to 1 gram every 6 to 8 hours. 'Ancef' has been administered in dosages of 6 grams per day in serious infections such as endocarditis.

'Ancef' may be used in patients with reduced renal function with the following dosage adjustments: Patients with a creatinine clearance of 55 ml./min. or greater or a serum creatinine of 1.5 mg. % or less can be given full doses. Patients with creatinine clearance rates of 35 to 54 ml./min. or serum creatinine of 1.6 to 3.0 mg. % can also be given full doses, but dosage should be restricted to at least 8-hour intervals. Patients with creatinine clearance rates of 11 to 34 ml./min. or serum creatinine of 3.1 to 4.5 mg. % should be given 1/2 the usual dose every 12 hours. Patients with creatinine clearance rates of 10 ml./min. or less or serum creatinine of 4.6 mg. % or greater should be given 1/2 the usual dose every 18 to 24 hours. All reduced dosage recommendations apply after an initial loading dose appropriate to the severity of the infection.

In children, a total daily dosage of 25 to 50 mg. per kg. (approximately 10 to 20 mg. per pound) of body weight, divided into three or four equal doses, is effective for most mild to moderately severe infections. Total daily dosage may be increased to 100 mg. per kg. (45 mg. per pound) of body weight for severe infections. Since safety for use in premature infants and in infants under one month has not been established, the use of 'Ancef' in these patients is not recommended.

For additional information on dosage and administration, see package literature or contact your SK&F Representative.

HOW SUPPLIED

Ancef (sterile cefazolin sodium, SK&F) – supplied in vials equivalent to 250 mg., 500 mg. or 1 gram of cefazolin; in Overfill ("piggyback") Vials for intravenous admixture equivalent to 1 gram of cefazolin; and in Pharmacy Bulk Vials equivalent to 5 grams or 10 grams of cefazolin.

Smith Kline & French Laboratories
Division of SmithKline Corporation, Philadelphia, Pa.
SK&F

American Journal of Obstetrics and Gynecology

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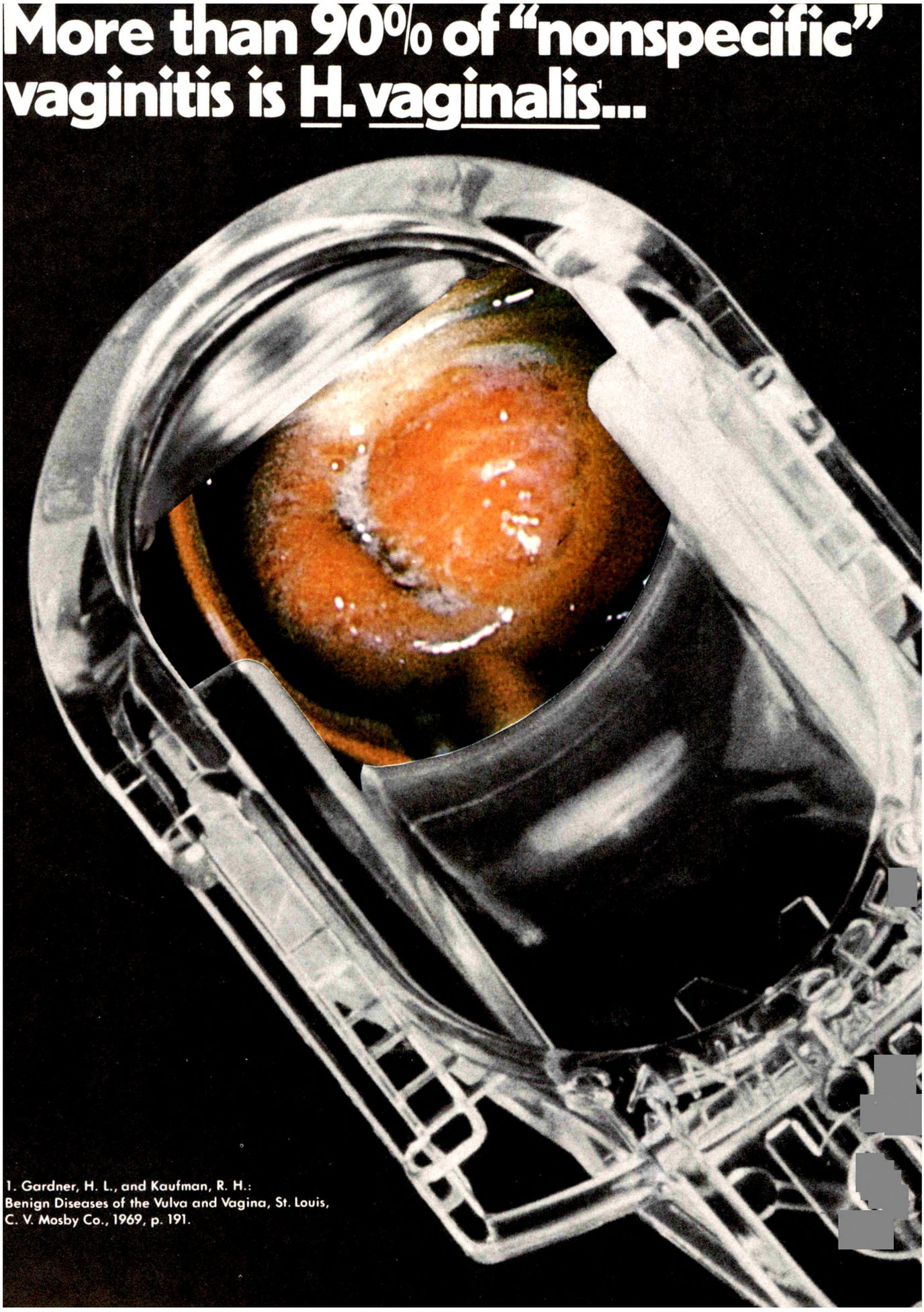
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More than 90% of "nonspecific" vaginitis is H. vaginalis[1]...



1. Gardner, H. L., and Kaufman, R. H.:
Benign Diseases of the Vulva and Vagina, St. Louis,
C. V. Mosby Co., 1969, p. 191.

...and specific for H. vaginalis is

Sultrin TRIPLE SULFA CREAM

TRADEMARK

Effective

Prompt symptomatic relief

Marked clinical response often within days

Effective in H.V. vaginitis occurring concomitantly with vaginal trichomoniasis and/or candidiasis

...also available in convenient vaginal tablets

Composition: SULTRIN Cream contains sulfathiazole 3.42%, sulfacetamide 2.86%, and sulfabenzamide 3.7%, and urea 0.64%. Each SULTRIN Tablet contains sulfathiazole 172.5 mg, sulfacetamide 143.75 mg, and sulfabenzamide 184.0 mg.

Indications: SULTRIN Cream and Tablets are indicated for treatment of *Haemophilus vaginalis* vaginitis. They may also be used as a deodorant for saprophytic infection following radiation therapy.

Contraindications: Sulfonamide sensitivity and kidney disease.

Dosages: SULTRIN Cream: One applicatorful intravaginally twice daily for 4 to 6 days. The dosage may then be reduced one-half to one-quarter. SULTRIN Tablets: One tablet intravaginally before retiring and again in the morning for ten days. This course may be repeated if necessary.

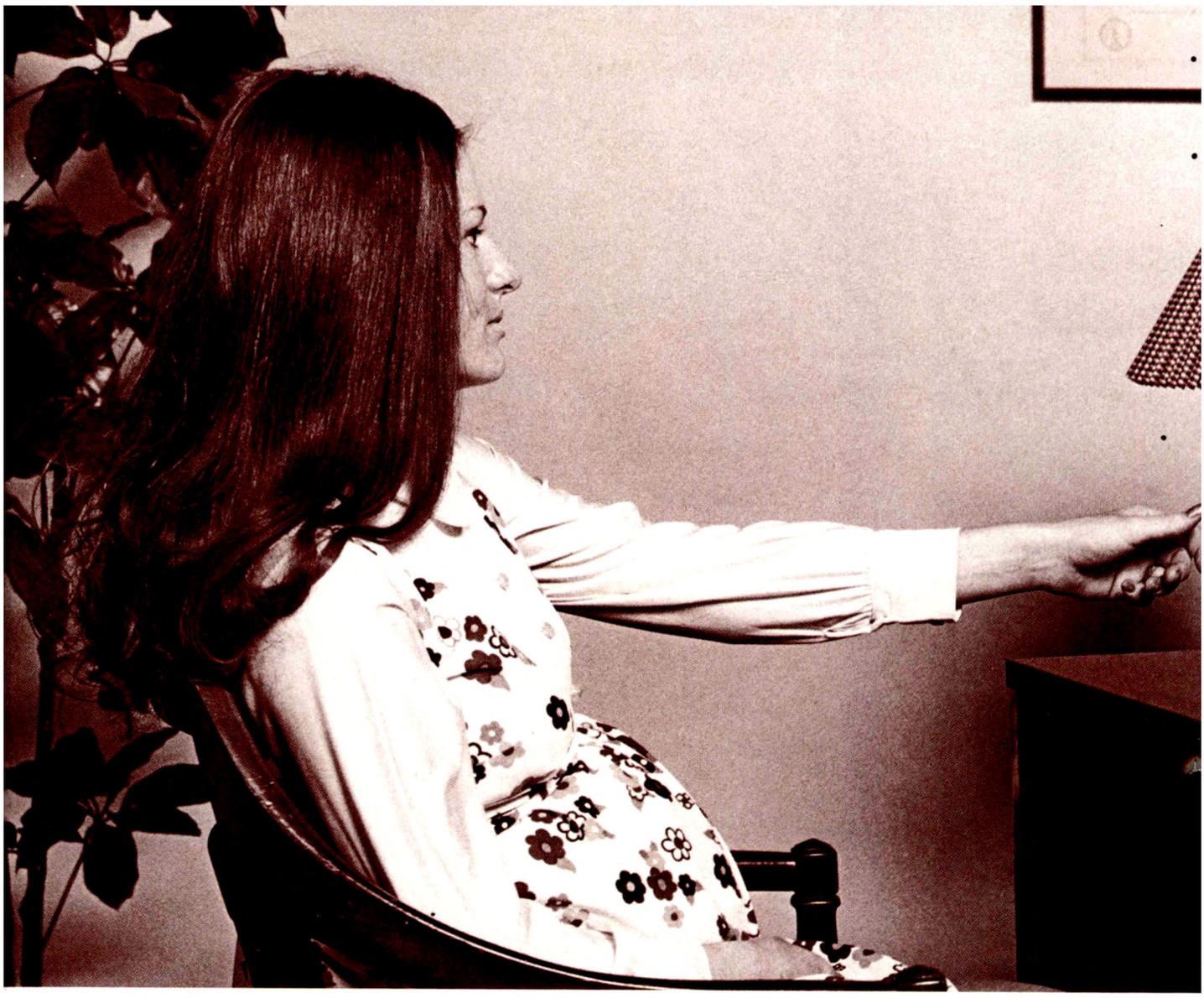
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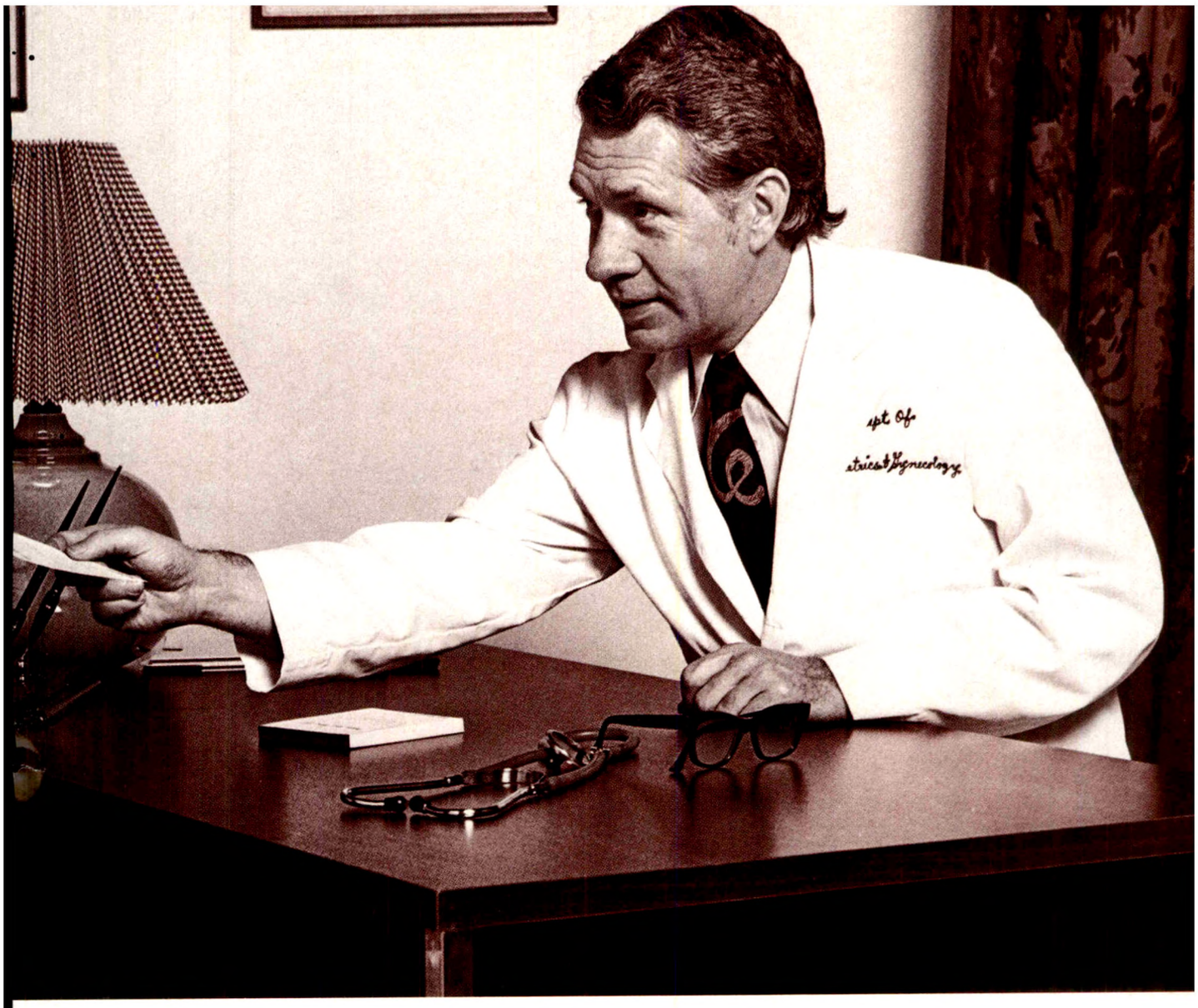
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- Each product contains the recommended*800 mcg. of Folic Acid for pregnancy.
- Each product contains 525 mg. of Ferrous Sulfate in controlled-release form to minimize gastric upset—an important consideration in pregnancy.
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*Federal Register, 38:20737, Aug. 2, 1973.



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Indications: For the treatment of iron deficiency and prevention of concomitant folic acid deficiency in non-pregnant adults. Also indicated in pregnancy for the prevention and treatment of iron deficiency and to supply a maintenance dosage of folic acid. **Contraindications:** Pernicious anemia. **Warnings:** Folic acid alone is improper therapy in the treatment of pernicious anemia and other megaloblastic anemias where vitamin B_{12} is deficient. **Precaution:** Where anemia exists, its nature should be established and underlying causes determined. Fero-Folic-500 contains 800 mcg. of folic acid per tablet. Folic acid especially in doses above 1.0 mg. daily may obscure pernicious anemia, in that hematologic remission may occur while neurological manifestations remain progressive. **Adverse Reactions:** The likelihood of gastric intolerance is remote. If such should occur the tablet may be taken after a meal. Allergic sensitization has been reported following both oral and parenteral administration of folic acid.

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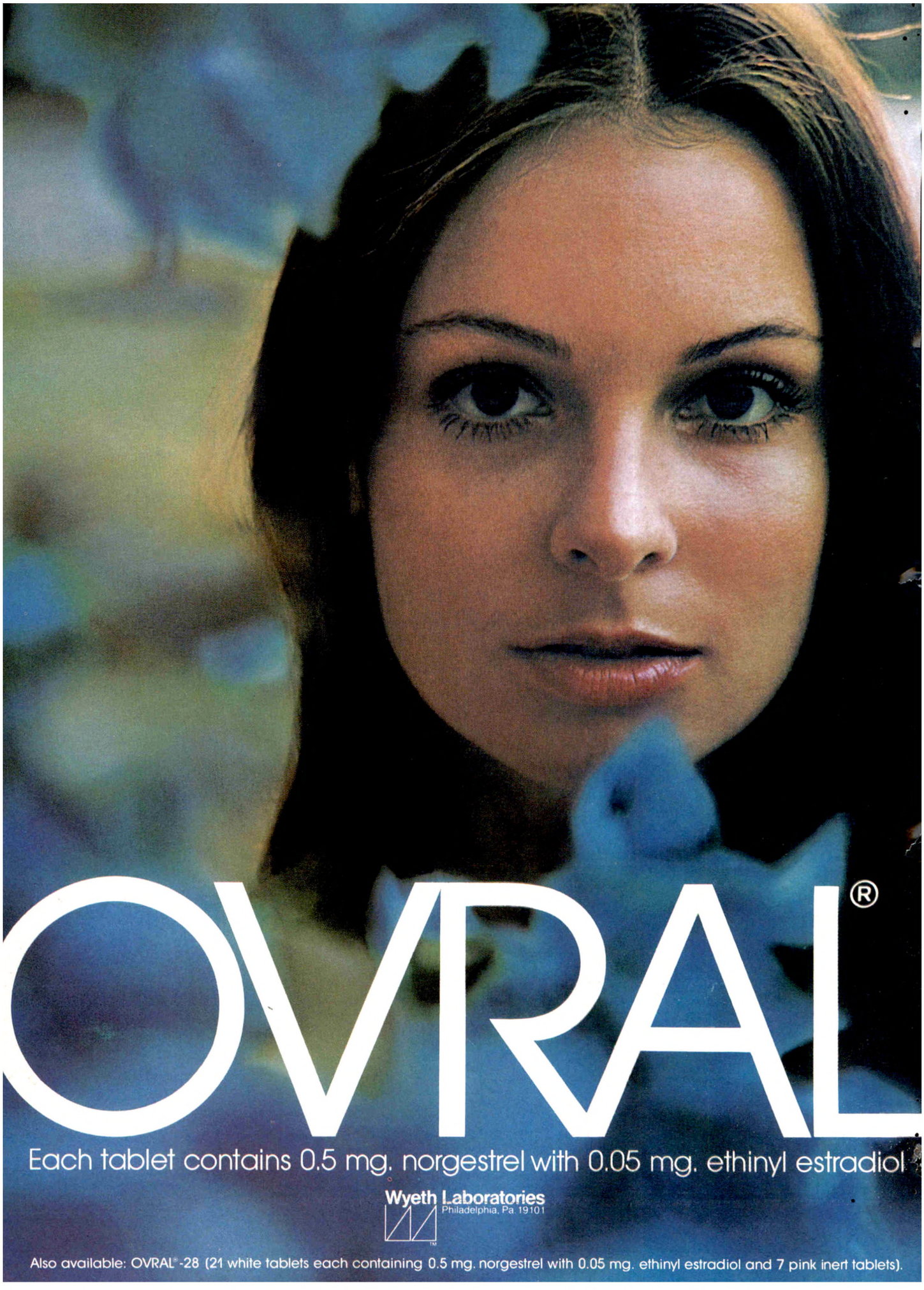
Plus the B-Complex

Niacinamide	30 mg.
Calcium Pantothenate	10 mg.
Vitamin B_1 (Thiamine Mononitrate)	6 mg.
Vitamin B_2 (Riboflavin)	6 mg.
Vitamin B_6 (Pyridoxine Hydrochloride)	5 mg.
Vitamin B_{12} (Cyanocobalamin)	25 mcg.

Plus 800 mcg. Folic Acid

Indications: In non-pregnant adults, for the treatment of iron deficiency and prevention of concomitant folic acid deficiency where there is an associated deficient intake or increased need for the B-complex vitamins. Also indicated in pregnancy for the prevention and treatment of iron deficiency where there is a concomitant deficient intake or increased need for B-complex vitamins (including folic acid). **Contraindication:** Pernicious anemia. **Warnings:** Folic acid alone is improper therapy in the treatment of pernicious anemia and other megaloblastic anemias where vitamin B_{12} is deficient. **Precaution:** Where anemia exists, its nature should be established and underlying causes determined. Iberet-Folic-500 contains 800 mcg. of folic acid per tablet. Folic acid especially in doses above 1.0 mg. daily may obscure pernicious anemia, in that hematologic remission may occur while neurological manifestations remain progressive. **Adverse Reactions:** The likelihood of gastric intolerance is remote. If such should occur, the tablet may be taken after a meal. Allergic sensitization has been reported following both oral and parenteral administration of folic acid.

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CLINICAL OPINION

This section reports opinion on the handling of clinical situations, i.e., the clinical diagnosis and management of certain disease entities. Papers should range from eight to twenty typed pages, including illustrations, tables, and figures which clarify the author's management. References are limited to six citations. Mail to Frederick P. Zuspan, M.D., Editor.

Unresolved issues in the first five years of the rubella immunization program

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Despite extensive use of attenuated rubella vaccine during the past five years, the degree of fetal risk in susceptible women inoculated early in pregnancy is still an unresolved issue. Although there is considerable evidence of chronic vaccine virus infection of the products of gestation in aborted fetuses, congenital defects have not yet been reported in the newborn infants of susceptible vaccinated pregnant women. The normal findings in the newborn infant can be misleading with respect to the safety of the vaccines in early pregnancy, because they represent only a small selected group of reported cases. The immediate need is for more complete reporting of all relevant cases wherever they occur in order to determine without many more years of delay whether the fetal risk has public health significance or is so low as to be of negligible importance.

SOME OF THE problems encountered in the control of congenital rubella (German measles) during the first five years of the immunization program require review and analysis. During this period, more than 50 million doses of rubella vaccines were distributed in the United States alone.[1] Unlike that for other vaccination programs, the target population for large-scale immunization in the United States has not been those at risk, susceptible women of childbearing age, but the age group from one to twelve, those who will require protection a decade or two later.[2] This ambiguous approach to the prevention of congenital rubella has been controversial since the inception of the program.[3–5] It was deemed necessary because the vaccine, though attenuated, consists of live virus capable of infecting the products of gestation in susceptible

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Table I. Frequency of vaccine virus isolated in fetal tissue

Reference	*Total*	*Positive*	*Negative*	*Infected tissue**
Preabortion vaccine inoculation				
Furukawa and associates[13]	14 (7)	0	14	
Vaheri and colleagues[14]	24 (24)	1	23	Kidney
Katz and co-workers[15]	2 (2)	0	2	
Inadvertent vaccine inoculation				
Fleet and associates[16]	9 (1)	1	8	Eye
Ebbin and colleagues[19, 22]	8 (2)	1	7	Bone marrow
Larson and co-workers[18]	5	0	5	
Total	62 (36)	3	59	

Numbers in parentheses are the numbers of known susceptible cases included in the total.

*Two additional positive cases (eye, kidney) were reported by the CDC from an unspecified number examined.[20]

Table II. Outcome of pregnancy following rubella vaccination shortly before or after conception*

Preinoculation Immune status	*Total*	*Spontaneous abortions*		*Induced abortions*		*Live births*		*Defective newborn infants*
		No.	*%*	*No.*	*%*	*No.*	*%*	
Susceptible	51	3	5.9	25	49.0	23	45.1	0
Unknown	239	12	5.0	112	46.9	115*	48.1	0†

*Based on data collected by the CDC, Atlanta, Georgia.[26]

†Only about 15 per cent of the unknown immune group or 17 women were probably susceptible before inoculation, according to expectancy in the general population.[26, 41]

women. The validity of the present policy on mass immunization of the childhood population depends on two interrelated questions that remain to be resolved: (1) Of what value is immunizing the prepuberty population as a measure to control contact spread and rubella outbreaks? (2) What is the degree of fetal risk in susceptible adolescents and adults who have been inoculated early in pregnancy?

The large-scale inoculation of children from one to twelve years of age was said to be of value in preventing rubella for several reasons. Most children were susceptible and could be readily inoculated on a large scale without the need of testing for susceptibility. Also, they were the primary source of infection for susceptible household contacts of childbearing age. As the main disseminators of infection, their mass immunization was considered essential to the prevention of outbreaks, according to the concept of herd immunity. However, field experience soon showed that the mass inoculation of young children could not be relied upon to prevent rubella outbreaks and infection of susceptible contacts. Epidemics affecting all susceptible subjects were reported in military units, even though about 85 per cent of the exposed population were found to be immune in serologic surveys.[6, 7] In civilian populations, large-scale inoculation of prepuberty children several months before an outbreak only served to protect that age group but not older girls of childbearing age.[8] Similar experiences have been reported in college outbreaks of rubella affecting large numbers of female students.[9]

The lack of protection for those most in need of the vaccine at the time of an outbreak is an inevitable outcome of a mass vaccination program that at present is geared primarily to prepuberty youngsters. This anomalous situation in public health practice could be justified partly on the grounds that any large-scale inoculation program for children over age 12 would necessarily include many susceptible out-of-wedlock pregnant teen-agers. Their number has increased in recent years, and their pregnancies are generally concealed in the first trimester when the fetus is most vulnerable to rubella virus infection. However, the nature of the ill effect to be expected in any large-scale inoculation program for young teen-agers with attenuated virus strains has remained largely speculative, because the nature and extent of fetal damage in susceptible inoculated pregnant women have not been well defined.

Current concepts on the degree of fetal risk in inoculated pregnant women derive from the high incidence of congenital malformations following maternal rubella in the first trimester and the necessity of using live virus strains in order to induce an adequate

immune response.[10–12] The infectivity of the vaccine virus for the products of gestation and the nature of the pathologic reactions were readily demonstrable in studies on susceptible women scheduled for abortions and on aborted fetuses and stillborn infants following inadvertent inoculation during pregnancy.[13–19] The frequency of demonstrable vaccine virus infection of the placenta and decidua was found to be about 25 per cent in susceptible cases.[14] On the other hand, considerable difficulty has been encountered in isolating vaccine virus from fetal tissue in susceptible cases. As shown in Table I, virologic studies were either negative or positive in single cases only, according to results reported in preabortion inoculated women and in women who were inadvertently inoculated while pregnant by six groups of investigators. In their combined series of 62 cases (36 known susceptible subjects), there were 3 positive fetal cases (eye, kidney, bone marrow). Two additional positive cases (fetal eye and kidney) have been reported by the Center for Disease Control (CDC) out of an unspecified number of cases in its own series of fetal tissue examinations.[20] The low frequency of positive fetal tissue has been attributed speculatively to the very small amounts of viable vaccine virus in its tissues and to failure to process specimens promptly and properly.[21] Evidence of chronicity of fetal infection has been reported in isolation of vaccine-like virus from the bone marrow 94 days after inadvertent inoculation in one case and from the eye of a 13 week fetus following maternal inoculation seven weeks before conception (described below).[16, 22]

Because of the hazards of vaccine virus infection of the fetus, release of the vaccine for general public use was not in accord with governmental safety requirements. Nevertheless, licensure of the vaccine was justified on at least two counts. First, vaccine virus was recommended on a large scale for prepuberty children only and on an individual basis for susceptible women, providing they would not become pregnant for two or three months.[2] Second, the risk of transmitting vaccine virus from inoculated subjects to susceptible pregnant contacts by means of infected nasopharyngeal secretions was rare.[11, 23–25] Subsequent experience soon showed that the contraindications to use of the vaccine during pregnancy were either disregarded or overlooked. Women either were unaware of being pregnant at the time of inoculation or they became pregnant soon thereafter despite precautions. In either instance, inoculation shortly before or after conception often led to an induced abortion or to months of anxiety over the outcome for those who went to term.

Specific information on the outcome of gestation for those inoculated early in pregnancy has been collected on a nationwide basis by the CDC.[20, 26, 27] A recent rubella surveillance report by the CDC describes results obtained on 300* women who were "inadvertently" vaccinated from 8 weeks before to 16 weeks after conception during 1970 to 1974.[26] Their distribution by preimmune status in serologic tests was unknown in 239 cases; 51 were susceptible and 10 were immune. In the small immune group, fetal defects were not expected and none was observed. The outcome in the other two groups consisting of 290 cases is shown in Table II by number of fetal deaths and live births. Fetal deaths occurred in about half the cases in each group mainly by "therapeutic" abortion. Since about 85 per cent of women in the United States are probably immune to rubella, most of the many induced abortions in the unknown immune group were unnecessary and could have been avoided by serologic testing before inoculation.

In about 45 per cent of the reported cases, gestation was not interrupted before term, and data are now available on the condition of 138 newborn infants following maternal rubella vaccination in the United States (Table II). There was no evidence of congenital defects in any of the live-born infants and no serologic evidence of congenital infection in 37 infants tested.[26] Since only susceptible women are actually at risk, the negative findings properly apply only to the 23 cases in the known susceptible group and to an estimated expectancy of about 15 per cent or 17 additional presumably susceptible cases in the unknown immune group, for a total of 40 cases (Table II). These favorable results seem to indicate that the fetal risk may be less common or less serious than expected from the chronicity of vaccine virus infection of the fetus and the high degree of susceptibility of fetal tissue to rubella virus. On the other hand, the reported outcome in the newborn infant may be very misleading because the number of cases is so small and probably is not representative of the total population at risk because of underreporting.

The histopathologic findings observed in aborted cases represent direct evidence of fetal injury, the nature and outcome of which are difficult to ascertain, because the process is interrupted at an early stage by the induced abortion and the cause is usually not confirmed by virologic studies. The reported tissue changes consist mainly of cellular infiltrations in the decidua and placenta and of damage to the fetus in a single case which affected the eyes.[16–19]

The cellular lesions represent initial response to

*Excluded are 17 additional cases in which the outcome of pregnancy was unknown.

vaccine virus infection or to some other cause and are terminated before the nature of the final outcome can be determined. Consequently, the prospect that such changes can lead to major congenital malformations is only conjectural. In the report on pathologic changes in the fetal eye, evidence implicating vaccine virus is described which was "consistent with congenital infection."[16] The histologic abnormalities consisted of pyknotic nuclei and cytoplasmic vacuoles in the fiber cells of the lens nucleus and "degenerative changes with liquification" in the lens cortex. In addition, "particles resembling rubella virus were seen on electron microscopy," and an interfering agent was isolated from the eye with "in vitro growth characteristics similar to those of vaccine virus." The abnormal findings resembled the early changes noted in wild virus infection.[28] They were observed in a fetus from a 25-year-old patient inoculated about 7 weeks before conception and aborted after 13 weeks' gestation. Since the abortion interrupted the course of the pathologic process, there is no way of knowing for sure whether it would have progressed to the formation of a nuclear cataract characteristic of congenital rubella. Even in natural rubella infection, the development of congenital cataracts and other major malformations cannot be determined "with assurance" from histopathologic changes in the aborted fetus.[28] Consequently, in search for conclusive evidence on the frequency of significant fetal damage, reliance should be placed on the occurrence of vaccine virus–induced congenital defects in the newborn infants of susceptible mothers inoculated early in gestation.

Such bona fide cases have not yet been reported in the United States. The experience in other countries appears to be similar to that described for the United States, according to a review of the available literature and correspondence with various investigators and with the Ministries of Health in Europe, South America, Mexico, Canada, Australia, New Zealand and Israel. Until authenticated cases are described, there seems to be no reliable way of confirming the teratogenic effects of rubella vaccines. The normal newborn findings indicate that the fetus was either not infected, as substantiated by negative serologic tests, or was infected but suffered no demonstrable major defect at birth, as occasionally observed in maternal rubella.[29] Since the reported number of normal cases is small and the duration of follow-up is short, much more time will be needed to determine whether the fetal risk is serious enough to be of public health significance or is so low as to be of negligible importance.

Accurate determination of fetal risk is an elusive problem not only because of incomplete case reporting, short-term follow-up, and unknown preinoculation immune status of inoculated mothers but also because of ethical considerations forbidding studies on randomly allocated inoculated and uninoculated susceptible women shortly before or after conception. In the absence of well-designed prospective studies, the risk cannot be determined reliably because the study population or the number of susceptible inoculated women is unknown. Furthermore, because of incomplete notification, the reported number of newborn infants represents a highly selected group of only about 10 cases per year in the United States and a smaller number elsewhere.[30–32] At this rate, it would take almost a decade to accumulate about 100 cases. More complete reporting would not only increase the number of cases but also make them more representative of the total at risk. By intensifying the search for cases both here and abroad, one may be able to determine without too many more years of delay the frequency of major malformations caused by rubella vaccines.

Until such meaningful data on the newborn infant become available, much can be done to reduce the number of unnecessary abortions by stressing the importance of determining rubella susceptibility before inoculation and the necessity of avoiding pregnancy for at least two months after inoculation. For those who do become pregnant, despite its contraindication, and prefer to go to term, the probable outcome of pregnancy remains uncertain for the present. In these cases, cord blood should be routinely tested for serologic evidence of congenital infection. The number of susceptible subjects reaching the childbearing age can be reduced by developing acceptable programs for large-scale inoculation of premenarchial schoolgirls who were not immunized in early childhood.[33] In addition, the size of the susceptible adult population can be decreased with minimal risk by inoculating seronegative postpartum women within 48 hours after delivery when the likelihood of conception is quite low for at least a month, even without precautions.[34] Where feasible, premarital and prenatal tests for rubella antibodies should be routine, and serologic surveys should be undertaken in other postpubertal groups.[35] If vaccination is recommended, the use of suitable informed consent provisions would be helpful to patient and physician.

The ultimate solution to the problem of preventing abortions, malformations, and the anxieties associated with rubella depends on the continued search for safer specific vaccines and other effective prophylactic measures. Further attenuation of currently used vac-

cine strains by additional tissue culture passage would increase their safety but reduce their immunogenicity or effectiveness. An obvious approach is to search for less teratogenic wild strains. Such strains are said to be prevalent in Japan where the incidence of rubella embryopathy is reported to be very low despite widespread epidemics.[36] Their low teratogenicity, if confirmed, may be related to their capacity to induce high levels of interferon, according to comparative studies in placental cell cultures.[37] Preliminary field trials in Japan and the United Kingdom indicate that a vaccine prepared from a Japanese strain attenuated by tissue culture passage (To-336) is as antigenic as current vaccines, and is nontransmissible to susceptible contacts.[38, 39] Its effect on the fetus has not yet been investigated. This crucial point will probably take a long time to clarify, because ethical restrictions limit such determinations to women inadvertently inoculated shortly before or after conception. Other measures that might be used to prevent or treat rubella are either not effective (e.g., drugs), or of questionable value (e.g., gamma globulin).

Whether interferon or its inducing agents can be used to protect the fetus of exposed or vaccinated mothers is in an early stage of investigation.[40]

In summary, there is no quick and easy solution to the problems arising from the current need to use live vaccine virus in the control of rubella (German measles). Despite its attenuation, the virus is capable of causing a chronic infection of the products of gestation and cellular reaction resembling an early lesion of wild virus infection. Whether the attenuated vaccine virus can cause major malformations characteristic of natural rubella has not yet been substantiated in studies of the newborn infant. Although the normal newborn findings are based on a small number of reported cases, they raise hopes that the risk of a major defect may be less frequent and less serious than expected. Because of sampling variation and incomplete reporting, these hopes may prove to be false. Whether they are real or false can be reliably ascertained only by an evaluation of fetal risk based on the outcome in the newborn infant. For this purpose, intensified and coordinated efforts will have to be made by official agencies and medical groups at more complete reporting of relevant newborn cases wherever they occur in the United States and in other countries. The results obtained should establish whether the risk from current vaccines is minimal or whether safer vaccines are needed for large-scale use in women of childbearing age.

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GYNECOLOGY

The effectiveness of two oral contraceptives in suppressing plasma androstenedione, testosterone, LH, and FSH, and in stimulating plasma testosterone-binding capacity in hirsute women

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The effectiveness of two oral contraceptives in suppressing plasma androstenedione (A), testosterone (T), LH, and FSH and in stimulating testosterone-estradiol–binding globulin (TeBG) was evaluated in 39 hirsute women. Twenty-seven hirsute women received norethindrone 2 mg.–mestranol 0.1 mg. (Group I) and 12 received norgestrel 0.5 mg.–ethinyl estradiol 0.05 mg. (Group II). Hormone assays were performed before treatment and at the end of 3 weeks of therapy. Ninety per cent of the women in both groups had an elevated plasma A and/or T. During treatment, plasma A, T, LH, and FSH were significantly reduced in both groups ($p < 0.01$). In Group I, 78 per cent of the women had a normal plasma A and T during treatment. In Group II, 83 per cent of the women had a normal A and T during treatment. There was a greater increase in TeBG in Group I ($p < 0.01$). It is concluded that these two oral contraceptives effectively suppressed the hyperandrogenism of most of the hirsute women.

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WE RECENTLY described the time-course of the suppression of plasma androstenedione (A), testosterone (T), LH, and FSH by a combination-type oral contraceptive containing norethindrone 2 mg. and mestranol 0.1 mg.* in seven hirsute women with

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**Ortho-Novum 2 mg., Ortho Pharmaceutical Corporation, Raritan, New Jersey, or Norinyl 2 mg., Syntex Laboratories, Inc., Palo Alto, California.*

Table I. Clinical data, plasma androstenedione (A), testosterone (T), LH, and FSH levels, and testosterone-binding globulin (TeBG) capacity before and during treatment with norethindrone 2 mg.–mestranol 0.1 mg. in 27 hirsute women (Group I)

Pt.	Age	Facial hair	Ovary size	A (ng./100 ml.)		T (ng./100 ml.)		TeBG (μg/100 ml.)		LH (mI.U./ml.)		FSH (mI.U./ml.)	
				Pre-R_x	R_x	Pre-R_x	R_x	Pre-R_x	R_x	Pre-R_x	R_x	Pre-R_x	R_x
1	31	2+	Normal	337	150	22	7	0.1	1.0	51	28	11	8
2	17	2+	Enlarged*	215	155	43	35	0.5	2.3	36	4	8	5
3	25	3+	Enlarged*	535	160	127	51	1.3	4.9	34	4	10	3
4	28	1+	Enlarged*	493	242	134	70	0.8	0.8	42	12	8	7
5	20	2+	Normal	258	162	55	37	1.6	5.4	5	3	3	2
6	21	1+	Normal	300	207	96	31	3.9	8.0	22	2	8	1
7	25	3+	Enlarged*	270	195	60	47	0.6	4.4	11	7	7	2
8	18	1+	Enlarged*	337	191	70	46	0.8	4.4	30	8	8	4
9	23	1+	Normal	208	167	77	58	0.5	4.7	9	3	6	3
10	24	1+	Normal	302	153	75	39	1.8	4.5	33	2	8	1
11	21	2+	Normal	223	120	44	36	0.6	2.5	76	15	8	2
12	23	1+	Normal	404	220	92	48	2.3	3.3	46	8	12	3
13	17	2+	Normal	319	134	47	38	0.8	1.8	14	2	11	2
14	19	2+	Enlarged*	302	221	39	47	0.4	4.7	25	22	8	6
15	19	3+	Normal	354	210	65	40	0.9	3.5	21	5	7	1
16	18	2+	Normal	289	101	81	32	1.0	1.6	57	14	8	5
17	28	4+	Normal	369	210	88	51	1.0	2.0	25	5	6	5
18	29	2+	Enlarged	530	189	85	41	1.6	2.7	11	8	7	1
19	21	1+	Enlarged	457	122	70	22	1.1	3.5	25	3	8	1
20	19	1+	Enlarged	310	98	111	26	0.8	1.6	55	6	9	4
21	24	1+	Normal	332	167	74	45	1.3	3.3	28	10	9	2
22	15	4+	Enlarged*	525	321	130	96	0.4	2.2	33	12	9	4
23	31	2+	Enlarged	488	192	78	64	1.7	6.4	29	14	9	3
24	29	3+	Normal	312	113	77	39	3.9	6.9	15	21	10	6
25	18	1+	Normal	387	260	59	41	1.5	3.8	14	6	7	5
26	27	1+	Enlarged	347	143	47	42	1.3	3.4	20	3	9	3
27	15	2+	Normal	272	114	72	35	0.3	3.6	25	5	10	3

*Proved polycystic ovaries.

polycystic ovarian disease.[1] After a refractory period, mean plasma A and T decreased progressively throughout the first 3 weeks of drug administration. Mean plasma A and T were suppressed 58 per cent and 65 per cent, and all elevated levels were reduced to normal. Suppression of A and T correlated temporally with the decrease in plasma LH, suggesting LH-dependent hyperandrogenism. The purpose of this study was to determine the degree of suppression of plasma A and T and the degree of stimulation of plasma testosterone-estradiol–binding globulin (TeBG) by norethindrone 2 mg.–mestranol 0.1 mg. or norgestrel 0.5 mg.–ethinyl estradiol 0.05 mg.* in 39 unselected hirsute women.

Materials and methods

The 39 hirsute women who participated in the study represented consecutive cases of hirsutism seen in our clinic, except for a few women who had to be excluded because they did not return for hormone assays while on therapy. Twenty-seven of the hirsute women received norethindrone 2 mg.–mestranol 0.1 mg. and are termed Group I. Twelve hirsute women received norgestrel 0.5 mg.–ethinyl estradiol 0.05 mg. and are termed Group II. There was no *a priori* basis for assigning the women to one or the other of the two therapeutic groups.

The clinical data of the women in Group I are listed in Table I and those of the women in Group II are listed in Table II. The women in Group I ranged in age from 15 to 31 years (mean, 22). The women in Group II also ranged in age from 15 to 31 years (mean, 22). Most of the women in both groups had mild to moderate hirsutism. The degree of hirsutism was graded on a 1+ to 4+ scale. A 1+ rating was used when there was increased hair only on the upper lip; 2+ for excessive hair on the upper lip and sideburns; 3+ for excessive hair on the upper lip, sideburns, and chin; 4+ was used to indicate a male-type beard. Cases 17 and 22 in Group I were virilized and were 4+ hirsute. Twelve of the 27 women in Group I had enlarged ovaries and seven of these had documented polycystic ovaries by direct visualization. Four of the women in Group II had enlarged ovaries. The majority of the

*Ovral, Wyeth Laboratories, Philadelphia, Pennsylvania.

Table II. Clinical data, plasma androstenedione (A), testosterone (T), LH, and FSH levels, and testosterone-binding globulin (TeBG) capacity before and during treatment with norgestrel 0.5 mg.–ethinyl estradiol 0.05 mg. in 12 hirsute women (Group II)

Pt.	Age	Facial hair	Ovary size	A (ng./100 ml.)		T (ng./100 ml.)		TeBG (μg/100 ml.)		LH (mI.U./ml.)		FSH (mI.U./ml.)	
				Pre-R_x	R_x	Pre-R_x	R_x	Pre-R_x	R_x	Pre-R_x	R_x	Pre-R_x	R_x
1	18	1+	Enlarged	301	188	78	32	0.4	1.3	34	8	6	4
2	15	2+	Normal	311	132	43	24	1.3	2.5	14	5	6	1
3	29	2+	Normal	272	121	118	27	1.1	2.2	22	3	8	2
4	31	1+	Normal	260	118	49	38	2.0	2.2	19	6	8	3
5	17	1+	Normal	372	335	98	67	1.1	1.4	22	14	6	7
6	28	3+	Enlarged	478	279	62	47	1.7	1.9	17	6	11	10
7	24	1+	Normal	535	138	106	35	1.7	2.1	24	5	6	3
8	20	1+	Enlarged	405	212	36	30	0.7	0.7	8	2	4	1
9	20	2+	Normal	555	265	55	35	0.8	2.2	70	4	7	1
10	17	1+	Enlarged	310	154	69	37	2.7	2.3	37	57	9	5
11	17	2+	Normal	408	302	69	40	0.3	0.8	24	10	6	5
12	22	1+	Normal	257	190	60	43	1.5	2.3	36	64	11	10

women in both groups had oligomenorrhea or amenorrhea.

Blood was obtained from the antecubital vein between 10 A.M. and 4 P.M. prior to therapy and again at the same time of day during the last part of the third week of the first cycle of oral contraceptive administration.

Plasma T was measured by radioimmunoassay as described by Furuyama and associates,[2] including preassay chromatography on alumina columns. There was significant cross-reactivity between dihydrotestosterone (DHT) and the T antiserum, but DHT was separated from T by the preassay chromatography. The interassay coefficient of variation of the T assay was ±8.9 per cent at the level of 56 ng. per 100 ml.

Plasma A was measured by radioimmunoassay in a manner similar to T but with an earlier eluate from the alumina columns.[1] There was significant cross-reactivity of androsterone with the A antibody, but it was separated from A by preassay chromatography. The interassay coefficient of variation of the A assay was ±9.5 per cent at the level of 568 ng. per 100 ml.

Plasma FSH and LH were determined by a two-stage, double-antibody radioimmunoassay technique similar to that of Morgan.[1, 3] The LH (LER-960) and the FSH (LER-1366) used for radioactive labeling and the antisera (human chorionic gonadotropin antiserum and human pituitary FSH antiserum absorbed with hCG) were furnished by the courtesy of the Endocrine Study Section of NIH. The second international reference preparation of human urinary menopausal gonadotropin (2nd IRP-HMG) furnished by the World Health Organization and LER-907, a human pituitary gonadotropin reference preparation furnished by the Endocrine Study Section of NIH, were used as standards. Assay results are expressed in terms of the 2nd IRP-HMG standard. The interassay coefficient of variation at 12 mI.U. per milliliter was ± 11 per cent for the LH assay and ± 12 per cent for the FSH assay.

Urinary 17-ketosteroids (17-KS) and urinary 17-ketogenic steroids (17-KGS) were determined by the method of Sobel and associates.[4] Urinary pregnanetriol (P-3) was determined either at Endocrine Science Laboratories, Tarzana, California, or Bioscience Laboratories, Van Nuys, California.

Plasma TeBG binding capacity was determined by the method of Rosner.[5] The interassay coefficient of variation of the TeBG assay was ± 3.3 per cent at the level of 3 μg per 100 ml.

Two-factor analysis of variance with repeated measures was used for statistical analysis.

Results

The A, T, TeBG, LH, and FSH levels before and during treatment of the women in Group I are listed in Table I and the same data on the Group II women are listed in Table II. Fig. 1 illustrates the mean pretreatment and treatment levels of plasma A, T, TeBG, LH, and FSH of the two groups of hirsute women.

Pretreatment. Nineteen Group I women and nine Group II women had an elevated plasma A. Pretreatment plasma A of the women in Group I ranged from 208 to 535 ng. per 100 ml. (mean ± S.E., 351 ± 19) and in Group II ranged from 257 to 555 ng. per 100 ml. (mean, 372 ± 31). Plasma A between 10 A.M. and 4 P.M. of 10 normal menstruating females ranged from 93 to 300 ng. per 100 ml. (mean, 195).

Twenty-one Group I women and nine Group II women had an elevated plasma T. Pretreatment plasma T in Group I ranged from 22 to 134 ng. per

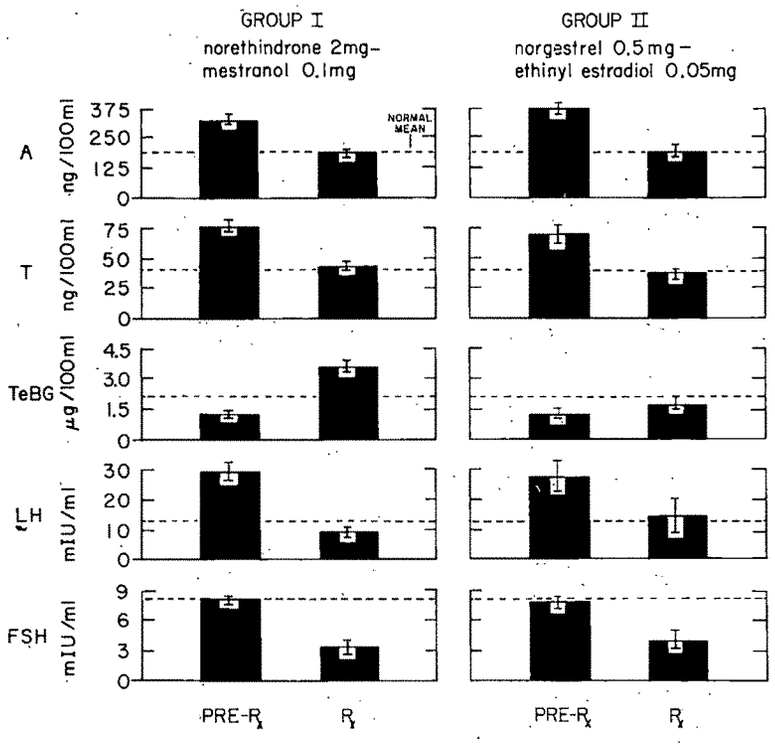


Fig. 1. Mean pretreatment (*pre-R_x*) and treatment (*R_x*) plasma levels of Δ^4-androstenedione (*A*), testosterone (*T*), testosterone-binding globulin (*TeBG*) capacity, *LH*, and *FSH* of the Group I and Group II hirsute women. Bars define the standard error of the means.

100 ml. (mean, 75 ± 5) and in Group II ranged from 36 to 118 ng. per 100 ml. (mean, 70 ± 8). Plasma T between 10 A.M. and 4 P.M. of 10 normal menstruating females ranged from 19 to 50 ng. per 100 ml. (mean, 39).

Plasma A and T were both elevated in 16 of the 27 Group I women. Plasma A and/or T were elevated in all but two of the Group I women. Plasma A and T were both elevated in seven of the 12 Group II women. Plasma A and/or T were elevated in all but one of the Group II women.

Fifteen Group I women and four Group II women had an abnormally low TeBG binding capacity. Pretreatment plasma TeBG binding capacity in Group I ranged from 0.1 to 3.9 µg per 100 ml. (mean, 1.2 ± 0.2) and in Group II ranged from 0.3 to 2.7 µg per 100 ml. (mean, 1.3 ± 0.2). Plasma TeBG binding capacity of 10 normal menstruating females ranged from 1.1 to 3.3 µg per 100 ml. (mean, 2.1).

Seventeen Group I women and five Group II women had an elevated LH level. Pretreatment plasma LH in Group I ranged from 5 to 76 mI.U. per milliliter (mean, 29 ± 3) and in Group II ranged from 8 to 70 mI.U. per milliliter (mean, 27 ± 5). Midfollicular plasma LH (day −7 from the ovulatory LH peak) of nine normal women ranged from 8 to 22 mI.U. per milliliter (mean, 13 ± 2).

Plasma FSH was within the normal range in all but one woman in each group. These two women had low levels of FSH. Pretreatment plasma FSH in Group I ranged from 3 to 12 mI.U. per milliliter (mean, 8 ± 0.3) and in Group II ranged from 4 to 11 mI.U. per milliliter (mean, 7 ± 0.6). Midfollicular plasma FSH (day −7 from the ovulatory LH peak) of nine menstruating women ranged from 5 to 12 mI.U. per milliliter (mean, 8 ± 1.4).

Eight women in Group I (Nos. 1, 3, 4, 5, 7, 8, 13, and 14) had elevated urinary 17-KS ranging from 1 to 4 mg. above the upper limit of the normal range. Seven women in Group I (Nos. 1, 3, 4, 7, 11, 13, and 16) had an increase in urinary P-3 of 1 mg. or less above the upper limit of the normal range. Four of the women who had elevated urinary 17-KS and/or elevated urinary P-3 had enlarged ovaries.

Treatment. There was a significant reduction of mean plasma A in both groups during treatment ($p < 0.01$). Treatment reduced mean plasma A of the women in Group I from 350 ± 19 to 175 ± 10 ng. per 100 ml. and reduced mean plasma A of the women in Group II from 372 ± 31 to 203 ± 22 ng. per 100 ml.

Plasma A was reduced in every individual of both groups and only one woman in Group I and two in Group II had a plasma A that remained elevated during treatment. The individual percentage decreases of plasma A in Group I ranged from 20 to 73 per cent (mean decrease, 48 ± 3) and in Group II ranged from 10 to 74 per cent (mean decrease, 45 ± 5).

There was a significant reduction in mean plasma T of both groups during treatment ($p < 0.01$). Treatment reduced mean plasma T of Group I from 75 ± 5 to 43 ± 3 ng. per 100 ml. and reduced mean plasma T of Group II from 70 ± 8 to 38 ± 3 ng. per 100 ml. Plasma T was reduced in every individual except one (No. 14, Group I), in whom the T level increased. Six Group I women and one Group II woman had an elevated plasma T during treatment. The individual percentage decreases of plasma T in Group I ranged from 11 to 77 per cent (mean decrease, 39 ± 4) and in Group II ranged from 17 to 77 per cent (mean decrease, 41 ± 6).

The degree of suppression of mean plasma A and T was not different in the two groups ($p > 0.05$). In Group I, 95 per cent of the elevated A levels and 71 per cent of the elevated T levels were reduced to within the normal range. In Group II, 78 per cent of the elevated A levels and 89 per cent of the elevated T levels were reduced to within the normal range. In Group I, 78 per cent of the women had a normal plasma A and T during treatment. In Group II, 83 per cent of the women had a normal A and T during treatment.

There was no significant difference in the degree of suppression of A and T between those who had normal urinary 17-KS and the others who had slightly elevated urinary 17-KS or between those who had a normal urinary P-3 and the others who had a minimally elevated urinary P-3 ($p > 0.05$).

Treatment significantly increased mean plasma TeBG binding capacity of the women in Group I from 1.2 ± 0.2 to 3.6 ± 0.3 μg per 100 ml. ($p < 0.01$). Mean TeBG of Group II was increased from 1.3 ± 0.2 to only 1.8 ± 0.2 μg per 100 ml. during treatment. There was a significantly greater increase in the TeBG binding capacity in the Group I women than the Group II women ($p < 0.01$). Plasma TeBG binding capacity was increased in 26 of the 27 women in Group I and in 10 of the 12 women in Group II. The maximum individual percentage increase in TeBG in Group I was 1,100 per cent (mean individual percentage increase, 323 ± 61) and in Group II the maximum percentage increase was 275 per cent (mean individual percentage increase, 82 ± 38).

There was significant reduction of mean plasma LH in both groups ($p < 0.01$). Treatment reduced mean plasma LH of Group I from 29 ± 3 to 9 ± 1 mI.U. per milliliter and reduced mean plasma LH of Group II from 27 ± 5 to 15 ± 6 mI.U. per milliliter. The maximum individual percentage decrease of plasma LH in Group I was 94 per cent (mean decrease, 65 ± 5) and in Group II was 94 per cent (mean individual percentage decrease, 47 ± 9).

There was a significant reduction of mean plasma FSH in both groups ($p < 0.01$). Treatment reduced mean plasma FSH of Group I from 8 ± 0.3 to 3.4 ± 0.4 mI.U. per milliliter and reduced mean plasma FSH of Group II from 7.3 ± 0.6 to 4.3 ± 0.9 mI.U. per milliliter. The maximum individual percentage decrease of FSH in group I was 88 per cent (mean, 58 ± 5) and in Group II was 86 per cent (mean, 44 ± 9).

Comment

Prior to the development of specific androgen assays, a large percentage of hirsute women were diagnosed as having constitutional hirsutism because they had normal urinary 17-KS. Following the development of specific A and T assays, most hirsute women were found to have elevated A and/or T plasma levels or production rates.[6-8] In the present study, plasma A and/or T were elevated in approximately 90 per cent of the hirsute women. In order to utilize A and T plasma levels to discriminate hyperandrogenic from normal women, several factors must be considered which are essential for properly defining the normal range. First, the assays must have specificity. If the A and T antisera are not entirely specific, preassay chromatography is necessary for reliable assays. Second, the time of day of the blood drawing must be considered in determining whether a plasma A value is normal or elevated, because of the diurnal rhythm of plasma A. Our studies indicate that in normal women there is a mean ± S.E. change of plasma A of 110 ± 11 ng. per 100 ml. throughout a 24 hour period which is synchronous with the change in cortisol, the major portion of the change occurring within 3 to 4 hours after the early morning peak.[9] Because the early morning peak of pituitary-adrenal activity occurs at different times in individuals, there is uncertainty whether a single blood specimen drawn in the early morning hours (6 A.M. to 9 A.M.) is at the time of the morning peak pituitary-adrenal activity or not. Therefore, a single plasma A value can be interpreted with greater confidence as to whether it is normal or high if it is drawn after 10 A.M. The time of the blood drawing is not as important a consideration in evaluating a

plasma T value, since the mean ± S.E. change of T throughout a 24 hour period is only 9 ± 3 ng. per 100 ml. in normal women.[9] However, the circulating TeBG binding capacity must be considered in utilizing plasma T levels to discriminate hyperandrogenic females from normal women. A normal total plasma T associated with an abnormally low TeBG binding capacity is presumptive evidence of hyperandrogenism, since circulating free T is likely increased in this circumstance.[10]

An important feature of the design of this study was that consecutive cases of hirsutism were studied. In this unselected group of hirsute women, the two oral contraceptives reduced plasma A and T to within the normal range in 78 and 83 per cent of the women. The data suggest that most of the women had a significant degree of LH-dependency of their hyperandrogenism, since the principal mechanism by which the contraceptive agents reduced plasma A and T was likely through suppression of LH. The fact that over one half (59 per cent) of the hirsute women in this study had an elevated pretreatment LH level also suggests LH-dependent hyperandrogenism.

Determination of A and T levels in ovarian and adrenal effluent blood indicates that the ovaries rather than the adrenals are more commonly the source of the increased A and T production in hirsute women.[11] The catheterization data are in keeping with the clinical observation that pituitary-ovarian suppression is generally more efficacious than pituitary-adrenal suppression in the treatment of hirsutism.[12] A pure adrenal source of hyperandrogenism is apparently uncommon in hirsute women.

Pituitary-ovarian suppression with a combination-type oral contraceptive should be performed in hirsute women with elevated A and/or T levels and normal or only minimally elevated 17-KS. Some of the earlier attempts to use oral contraceptives for the suppression of elevated A and T levels in hirsutism were disappointing, but this was likely due to the fact that the agents were not given long enough. The pituitary-ovarian axis is slower to suppress with sex steroids than the pituitary-adrenal axis is with glucocorticoids. The longer suppression time is due at least in part to the positive feedback effect of the estrogen content of the oral contraceptive on the secretion of LH. A study of the dynamics of the suppression of A and T in polycystic ovarian disease indicated that at least 3 weeks of norethindrone 2 mg.–mestranol 0.1 mg. therapy is required to suppress the pituitary-ovarian axis.[1] It is important to emphasize that maximal attainable suppression of LH, A, and T may not occur after 3 weeks of therapy in some hirsute women. The time required to evaluate the maximum degree of suppressibility of LH, A, and T by norethindrone 2 mg.–mestranol 0.1 mg. or norgestrel 0.5mg.–ethinyl estradiol 0.05 mg. in hirsute women has not been established. Consequently, hirsute women who have A and T levels that remain elevated after 3 weeks of therapy should have the levels determined again after two or three cycles of drug administration.

The choice of the suppressive agent may be critical. Estrogen alone is a poor suppressant of LH in hirsute women.[13] Plasma T is increased, not decreased, in hirsute women given norethindrone 1 mg.–mestranol 0.08 mg.[10] Further investigation is needed to evaluate the relative effectiveness of various progestin-estrogen combinations in suppressing LH, A, and T in hirsute women.

The reduction of plasma T in our patients during treatment is more impressive when the accompanying increase in TeBG binding capacity is also taken into consideration. The fact that T levels were decreased at a time when the TeBG binding capacities were increased indicates there was a reduction in T production, since T levels should rise when TeBG binding capacity increases if no change occurs in T production.

Norethindrone 2 mg.–mestranol 0.1 mg. induced a significantly greater increase in TeBG binding capacity than norgestrel 0.5 mg.–ethinyl estradiol 0.05 mg. The larger increase in TeBG binding capacity probably reflects the larger amount of estrogen in this agent. Furthermore, the antiestrogenic and/or androgenic activity of norgestrel may have partially counterbalanced the stimulatory effect of the ethinyl estradiol on TeBG synthesis.[14] Increasing TeBG binding capacity may be beneficial in and of itself in hirsute women because of the associated decrease in the amount of circulating free or biologically active T.[10] Although the degree of A and T suppression was comparable with the two contraceptive agents, the greater increase in TeBG by norethindrone 2 mg.–mestranol 0.1 mg. may make it a preferable agent in the treatment of hirsutism.

It is noteworthy that the degree of suppression of A and T was no less among those hirsute women who had a minimal elevation of urinary 17-KS and P-3 as compared to those who had normal 17-KS and P-3. These women may have a combined adrenal and ovarian source of their hyperandrogenism. It should not be assumed that the reduction of plasma A and T by these combination-type oral contraceptives is due exclusively to decreased ovarian secretion. The cortisol secretion rate is decreased by approximately 30 per cent in normal women receiving norethindrone 2 mg.–mestranol 0.1 mg.[15] The decrease in cortisol

production apparently results from the decreased metabolic clearance rate of cortisol secondary to an increase in the circulating level of cortisol-binding globulin. The reduced cortisol secretion rate is probably accomplished through decreased ACTH secretion, although this point has not been documented by the measurement of plasma ACTH. It is not known whether the decreased cortisol production is associated with decreased adrenal androgen production. If there is decreased ACTH secretion, then a portion of the decrease in the plasma A and T may be due to reduced adrenal as well as ovarian secretion of A and/or T.

The suppression of A and T concomitant with the suppression of LH by sex steroids does not rule out an ovarian or adrenal tumor, since tumors of these glands can be LH-dependent. A stromal luteoma of the ovary and two cases of T-secreting adrenal adenomas have been noted to be gonadotropin dependent or responsive.[16–18] When the A level is over 1,000 ng. per 100 ml, or the T level is over 300 ng. per 100 ml., the possibility of an adrenal or ovarian tumor must be considered.[19] The location of a tumor is likely best determined by differential catheterization of the adrenal and ovarian veins and measurement of A and T in the glandular effluent blood, rather than by suppression or stimulation studies.

Although an objective parameter was not used in this study to measure the effect of these contraceptive agents on hirsutism, approximately 60 per cent of the 20 hirsute women of this study who have received norethindrone 2 mg.–mestranol 0.1 mg. for more than 6 months subjectively reported a decrease in the rate of hair growth and/or a decrease in the amount of facial hair.

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Gonadotropin-releasing hormone radioimmunoassay and its measurement in normal human plasma, secondary amenorrhea, and postmenopausal syndrome

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A sensitive and specific double antibody radioimmunoassay for gonadotropin-releasing hormone (GnRH) has been developed for measurement in ethanol extracts of human plasma. Iodinated hormone was prepared with the use of the chloramine-T method, and antibodies were developed in rabbits over a six-month period with a GnRH synthetic copolymer immunogen. A Scatchard plot revealed at least three species of antibody. The assay can measure conservatively at the 5 pg. per milliliter level and shows no cross-reactivity with other available hypothalamic and pituitary hormones. The releasing hormone was quantitatively recovered from human plasma with immunologic identity to native hormone. Unextracted plasma could not be used because of nonspecific displacement. The measurement of GnRH in individuals receiving 100 μg of intravenous bolus infusions of the synthetic decapeptide show extremely elevated values with two half-lives: one of two to four minutes and another of 35 to 40 minutes. In our experiments, we have found measurable GnRH in patients with secondary amenorrhea and at the midcycle in normal women. In the normal cycling woman during the follicular and luteal phases, GnRH was undetectable. In postmenopausal women with extreme hypoestrogenism and markedly elevated luteinizing hormone values, GnRH was also undetectable. No bursts of GnRH could be detected in normal men when sampled every ten minutes over a two-hour period and every two hours throughout the day.

THE STRUCTURAL IDENTIFICATION and synthesis of luteinizing hormone–follicle-stimulating hormone–releasing hormone (GnRH) from porcine hypothalamus[1] has allowed evaluation of the physiology and control of the secretions of pituitary gonadotropins. However, the elucidation of significant information depends to a major extent on a sensitive and specific assay for GnRH itself. Several groups have already reported an immunoassay for GnRH,[2–4] while Malacara and associates[5] have developed significant biological and clinical data with the use of a bioassay for evaluation of feedback control of this hormone. We would like to report a sensitive and specific radioimmunoassay for GnRH and measurement in normal human men and women as well as in clinical conditions such as postmenopausal syndrome and secondary amenorrhea and relate this to control of gonadotropin secretion.

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**In partial fulfillment of the doctoral degree.*

Material and methods

Synthetic GnRH as well as a human luteinizing hormone (LH) immunoassay kit, human growth hormone, and a reference pituitary preparation of LH and follicle-stimulating hormone (FSH) (LER-907) were obtained from the National Institutes of Health, Hormone Distribution Section. Thyrotropin-releasing hormone (TRH) was obtained from Beckman Instrument Company and porcine adrenocorticotropic hormone (ACTH) from Calbiochem. Chromatographically pure human chorionic gonadotropin (HCG, 5,700 I.U. per milligram by Second International Reference Preparation for human menopausal gonadotropin) was

prepared in our laboratory from crude urine pregnancy preparations obtained from Organon (3,000 I.U. per milligram by Second Internation Reference Preparation for human menopausal gonadotropin).

A synthetic copolymer of glutamic acid, lysine, alanine, and tyrosine (GLAT) with a molecular weight of 30,000 in the molar proportion 0.36, 0.24, 0.35, and 0.05 was a kind gift of Jefferson Medical College, Department of Biochemistry. Sephadex media was obtained from Pharmacia Fine Chemicals, I^{125} from Amersham Searle, Freund's adjuvant from Difco, and egg albumin and rabbit gamma globulin from Pentex. Chloramine-T, sodium metabisulfite, benzidine, sodium azide, ethanol, and other salts and solvents were obtained from Fisher Scientific and were ACS grade or better. A Nuclear Chicago Model 1185 gamma counter was used to monitor radioactivity. Amino acid analysis was performed on a BioCal Model 200.

Coupling of GnRH to synthetic immunogen (GLAT). A synthetic copolymer was used for coupling rather than a biologically derived protein (albumin) to allow quantitation of bound GnRH. GnRH was covalently coupled to GLAT via bisdiazo reaction. GnRH (500 mg.) and GLAT (710 mg.) were dissolved in 2 ml. of sodium borate 0.3M, pH 9 (molar ratio GnRH:GLAT = 15:1). The diazobenzidine was prepared by addition of two 100λ aliquots five minutes apart from sodium nitrite, 400 mg. per milliliter in H_2O, to ice-cold benzidine, 30 mg. in 10 ml. of 0.05M HCl. Three 100λ aliquots of the reacted benzidine-HCl solution were then added to the GnRH mix, five minutes apart. After an additional 30 to 45 minutes in the cold the solution was applied to a G-25 fine column (2.0 × 30 cm.) and eluted with ammonium bicarbonate 0.05M, pH 7.8. The initial yellow protein peak at the void volume as per optical density 280 mμ was collected, lyophilized, and stored. Amino acid analysis revealed 6 moles of GnRH per mole of GLAT.

Immunization. New Zealand white rabbits, 2.5 kilograms, were given intramuscular injections of an emulsified mix of 0.5 ml. of Freund's complete adjuvant and 0.5 ml. of Krebs-Ringer buffer containing 100 μg of the GLAT-GnRH complex every three to four weeks for five months. Generation of workable antisera (titer > 1:1,000) was achieved between three to six months in several rabbits. Animals were bled every two to three weeks and plasma titered as described below.

Iodination. Iodination was carried out by the method of Hunter and Greenwood.[6] To 1 mc. of I^{125} was added 25 λ of 0.4M phosphate buffer, pH 7.5, followed by 2 to 5 λ of a 1 mg. per milliliter solution of GnRH (1 to 2.5 μg), followed by 5 λ of 3 mg. per milliliter chloramine-T solution and 60 seconds later by 250 λ of an 0.12 mg. per milliliter Sodium Metabisulfite solution. The GnRH, chloramine-T, and metabisulfite were all dissolved in 0.1M phosphate, pH 7.5. The iodinated material was then developed on a 0.9 × 75 cm. column of G-25 fine Sephadex and eluted with a 0.1M phosphate buffer, pH 7.5, containing 1 mg. per milliliter egg albumin and 0.1 per cent sodium azide. Approximately 200 tubes of 0.6 ml. fractions were collected, and 10 λ samples were aliquoted from each tube and radioactivity counted. Then 0.5 ml. of a 1/1,000 dilution of antibody was added to the same tubes, incubated at 20 degrees C. for 24 hours and precipitated with 0.5 ml. of saturated ammonium sulfate. Free GnRH I^{125} had less than eight per cent precipitation while bound GnRH or gamma globulin was quantitatively precipitated. Tubes were centrifuged after 30 minute incubation, supernatant removed, and the pellets resuspended in 1 ml. of 50 per cent ammonium sulfate. They were then recentrifuged for ten minutes, supernatant removed, and the pellets counted. Specific activity of immunologically active GnRH ranged from 60 to 500 μc per microgram or from 0.1 to 0.3 moles I^{125} per mole GnRH. This simple precipitation method could not be used in plasma because of nonspecific effects.

Standard curve. The displacement curve was generated in a total volume of 0.5 ml. by addition of GnRH I^{125}, 250 λ (approximately 5,000 counts per minute, 6 pg. of GnRH) in 0.1M phosphate buffer, pH 7.4, with 0.25 mg. per milliliter of carrier rabbit gamma globulin, ethylenediaminetetracetic acid (EDTA), 0.01M, and 0.1 per cent sodium azide). To this was added cold GnRH in a volume of 200 λ followed by 50 λ antibody in a 1 to 400 dilution (final running dilution of 1 to 4,000). Plasmas were worked up by extraction of 0.5 ml. with 1.5 ml. of ethanol followed by air drying of the supernatant ethanol extracts. This took approximately two hours, after which the residue was reconstituted in 200 λ of buffer to which GnRH I^{125} and antibody were added as above. The extraction of GnRH I^{125} by ethanol was routinely over 95 per cent, and thus no correction was made for extraction efficiency. After 24 hours incubation at room temperature, 100 λ of goat antirabbit gamma globulin was added to each tube and allowed to incubate overnight at four degrees C. The tubes were then centrifuged for 20 minutes at 3,000 r.p.m., supernatant removed by vacuum, and the pellets counted on the gamma counter. For the specificity studies, identical procedures were used with other hormones replacing unlabeled GnRH.

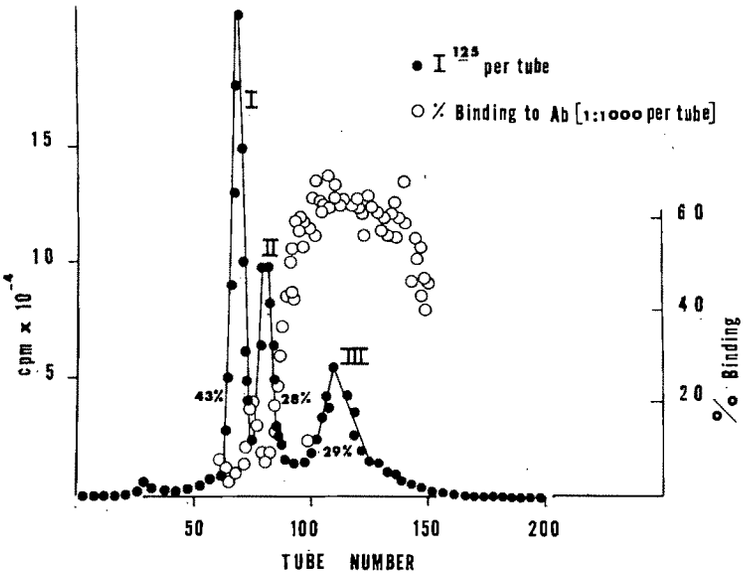


Fig. 1. Separation by Sephadex G-25 fine chromatography of GnRH iodination mix into I^{125} *(peak I)*, damaged GnRH *(Peak II)*, and immunologically active GnRH I^{125} *(peak III)*.

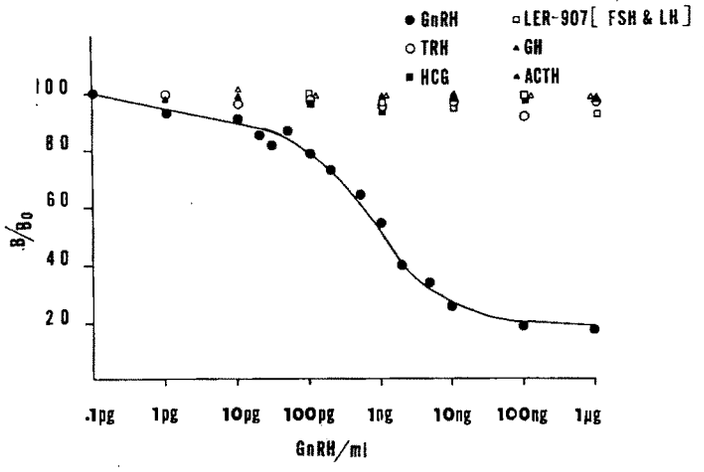


Fig. 2. Displacement of GnRH I^{125} from antibody with added GnRH or pituitary or hypothalamic hormones. Results are expressed as the ratio of amount bound at each point *(B)* to the initial point B_o (no added hormone).

LH immunoassay was performed by the double antibody method of Odell, Ross, and Rayford.[7]

Patient selection. Heparinized blood was collected randomly (10 A.M. to 3 P.M.), refrigerated within 15 minutes, spun down at 1,000g up to two hours later, and plasma stored at zero degrees C. until assay. Normal men (nine subjects) and women (eight subjects) were laboratory and medical school personnel (ages 22 to 38 and age 19 to 37, respectively). Normal female blood was drawn in both the follicular and luteal phases as well as midcycle as judged by basal body temperature and subsequently by LH rise in two patients. The patients with secondary amenorrhea (five subjects, age 20 to 28) had no history of drug use, weight loss, anorexia nervosa, significant psychologic disturbance or personality disorder, galactorrhea, diabetes, or hyperthyroidism. All were outpatients with amenorrhea of three to six months' duration with estrogen deficiency as judged by vaginal cytology. The postmenopausal women (eight subjects) were all nonhospitalized, ambulatory patients, ranging in age from 51 to 69. Half of the patients in this group complained of hot flashes. No patient was on any medication for up to two months prior to this study.

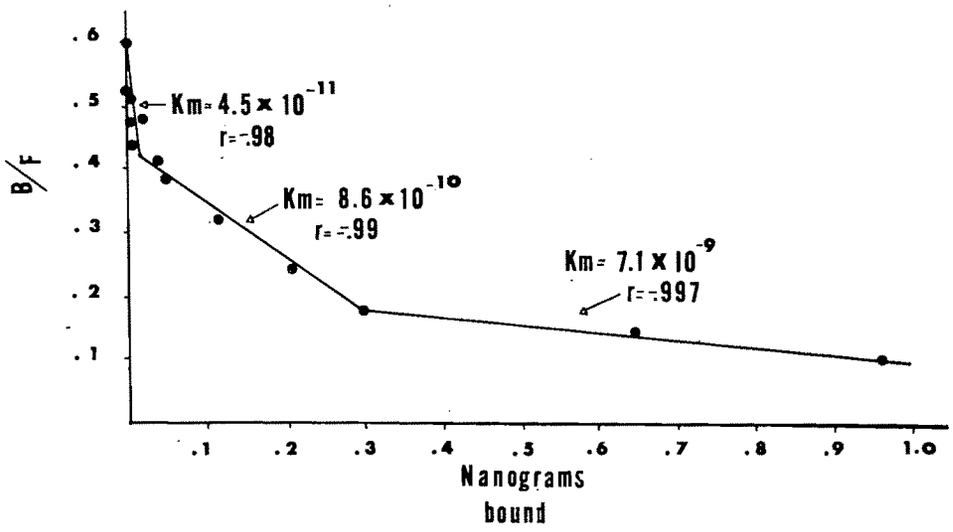


Fig. 3. Scatchard plot derived from Fig. 2. is expressed as ratio of antibody bound *(B)* and free *(F)* GnRH I^{125} versus nanograms bound per assay tube. *Km* is the dissociation constant between GnRH and antibody for each phase of displacement and *r* is the statistical correlation coefficient for the derived straight line. Three sets of antibody sites are presumed from this data.

Results

Iodination. Separation of the iodination mix on G-25 Sephadex fine gave three distinct peaks (Fig. 1). Peak 1 had identical chromatographic mobility as I^{125} on Whatman paper 3 MM with the use of a 0.1M phosphate buffer in a simple wick chromatography system.[8] Peaks 2 and 3 had identical mobility on wick chromatography, but only Peak 3 bound to antibody. Peak 1 was assigned to I^{125}, Peak 2 to damaged GnRH, and Peak 3 to GnRH I^{125} tracer with binding activity. Other investigators[9] have reported heterogeneity of iodinated GnRH and formation of "damaged" polymers. It should be stressed that our material has constant specific activity across the binding peak; that is, the counts bound per total mass of I^{125} per tube are identical. This is a major criterion of chemical purity, buttressing the fact that Peak 3 is immunochemically pure across all of its tubes without further purification necessary. This differs from the results of Arimura and associates[4] in which several ion-exchange purification methods were necessary to obtain more active material.

In some runs the efficiency of iodination was so good that the initial free iodide peak was absent, and only damaged and active GnRH was present. Slightly higher specific activity could be obtained if the column was developed at four degrees C. as opposed to room temperature (20 degrees C.), Approximately 20 tubes from Peak 3 were frozen at zero degrees C., thawed individually on the day of assay, and the remainder discarded. Iodinations were carried out at least once per month to maintain high specific activity. The efficiency of iodination (production of immunologically active material) ranged from 20 to 50 per cent of total iodide added.

Standard curve. Antibody from one animal at a titer of 1:4,000 (final dilution) bound 35 to 40 per cent of tracer with significant displacement with the use of unlabeled GnRH (Fig. 2). No displacement could be achieved with the use of TRH, HCG, LER-907 (a standard gonadotropin reference preparation containing LH and FSH) and human growth hormone, and ACTH.

The antibody thus had a significant degree of specificity. Cogeners of GnRH were not available for testing which could have provided significant further information on the sequence specificity of the antibody.* However, neither TRH nor other pituitary hormones cross-reacted. It can also be seen from the Scatchard plot in Fig. 3 that three distinct series of lines are generated with the use of the data in Fig. 2. This indicates that the antisera contain at least three distinct species of antibody which recognize GnRH over a 150-fold range in affinity. The sensitivity of the assay varied between less than 1 to 5 pg. per milliliter and the curves were easily reproducible.

Recovery. While these results indicated that our

*A total of eleven derivatives supplied by Parke, Davis & Co. and Abbott Laboratories have subsequently shown specificity to the C-terminal tripeptide amide (-Arginine-Proline-Glycine amide).

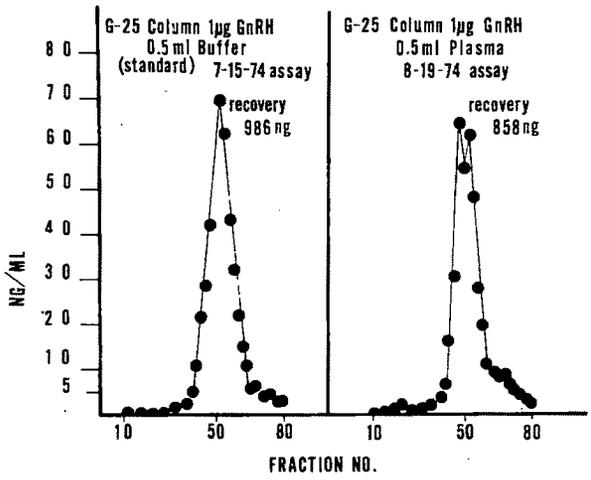


Fig. 4. G-25 fine chromatography of GnRH added to buffer or plasma to evaluate presence of binding proteins and recovery of added material.

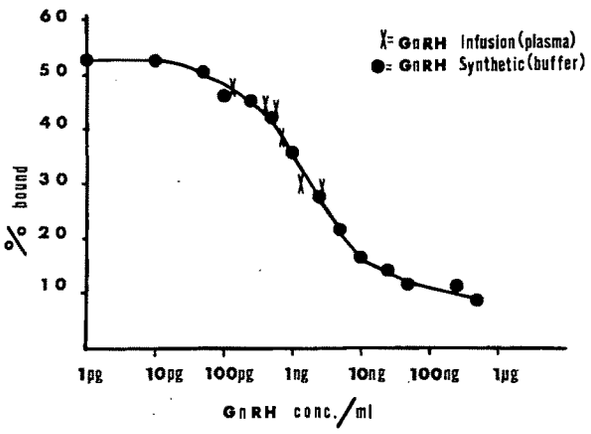


Fig. 5. Standard displacement curve with dilutions of plasma derived from a GnRH infusion to illustrate immunochemical identity.

system had immunologic specifity, two recovery experiments were performed to show identity between GnRH in plasma and buffer.

GnRH measured in unextracted dialyzed plasma still gave significantly high blank values in the nanogram range. Thus, it was important to establish that measured immunochemical GnRH had a chemical identity to synthetic GnRH to rule out measurement of several different cross-reacting species. A purposely large 1 µg load was added to plasma to saturate any carrier proteins and show any binding to higher molecular weight material. Sephadex G-25 chromatography was performed in the identical calibrated 0.9 × 60 cm. column to calculate not only recovery but evaluation of other immunologically active material from the elution position. As seen in Fig. 4, the plasma and buffer GnRH were quantitatively recovered from the column in identical positions without any evidence of binding to higher molecular weight components. The absence of multiple components in the chromatography experiments points to a species of immunologically measured GnRH in plasma with a molecular weight similar, if not identical, to synthetic GnRH.

In addition, immunologic identity of plasma hormones to unlabeled hormone in buffer was evident with the use of plasma from a GnRH infusion study containing 8 ng. per milliliter (Fig. 5). These diluted identically to the standard buffer curve. Thus, GnRH recovery and immunologic identity of plasma GnRH, as well as specificity, seems inherent to our system.

GnRH infusion. The measurement of GnRH in individuals receiving a 100 µg intravenous bolus infusion is shown in Fig. 6. The disappearance rate of GnRH is usually rapid with half-lives of approximately two to four minutes for one phase and 37 minutes for the second phase. These disappearance rates are so rapid, in fact, that considerable difficulty would be encountered in measuring small hypothalamic secretions. The 30 minute phase occurs after over 90 per cent of the material has been removed from plasma.

Patient data.

Plasma levels of GnRH in both normal men and women (follicular and luteal phases) were less than 5 pg. per milliliter (Fig. 7). In addition, no periodic secretion could be detected in normal men in samples drawn every two hours from 9:00 A.M. to 11:00 P.M. or every ten minutes over a two-hour period. Levels in postmenopausal women were undetectable. In contrast to this four of five individuals with secondary amenorrhea but normal LH had very significant measurable levels ranging from 6 to 26 pg. per milliliter. Two individuals on the day of ovulation as judged by basal body temperatures and LH rise also had elevated values.

Comment

GnRH can be measured conservatively in either buffer or plasma at the 5 pg. per milliliter level with this assay. Recovery of unlabeled material added to plasma was essentially quantitative and plasma GnRH diluted identically to the buffer curve. The immunologic identity, as well as recovery and specificity, define a workable assay. It should be stressed that GnRH cannot be measured in this system on unextracted plasma since significant nonspecific cross-reaction (that is, displacement) occurs which is dose related to the amount of plasma utilized. The normal of less than 5 pg. per milliliter becomes "1,000 t

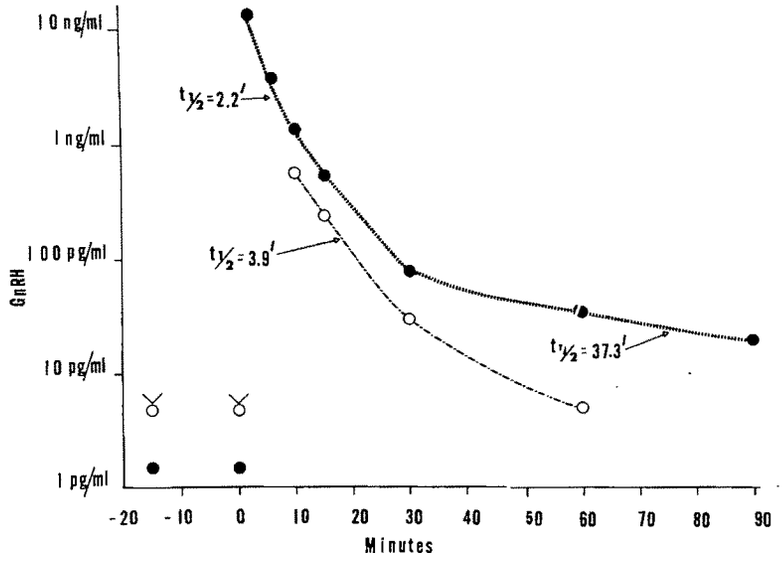


Fig. 6. GnRH infusion in two patients illustrating 1,000-fold rise from basal levels with rapid decrement. $t_{\frac{1}{2}}$ = half-life.

2,000" pg. even in GnRH free dialyzed plasma. Unlike TRH, however, which undergoes a time-dependent destruction in plasma,[10] GnRH is remarkably stable in plasma or serum at 20° to 25° C. for at least 48 hours and at 4° C. for 96 hours as judged by recovery of added unlabeled GnRH to plasma or serum.

GnRH is easily measured during bolus infusion studies with a two-component disappearance curve: one $T_{\frac{1}{2}}$ two to four minutes and the other 35 to 40 minutes. Because of the extremely rapid disappearance of the bulk of the material it would be extremely difficult to measure random samples even in acute GnRH bursts. Indeed, even with a massive 100 μg bolus infusion the return to normal levels is virtually complete at approximately 60 to 90 minutes.

Good evidence exists for a physiologic role for GnRH. Its rise is consistent with the midcycle LH surge as shown by us and others[11, 12] and the testicular atrophy shown in male rabbits immunized to GnRH.[4] Also, it has been shown that ovulation can be blocked in rats with the use of GnRH antiserum.[13] In our assay normal levels of GnRH are undetectable (less than 5 pg. per milliliter) in normal men and cycling women, except during the midcycle. These results are in agreement with those of Jeffcoate and co-workers[2] (less than 20 pg. per milliliter) and Arimura and associates[4] (less than 10 pg.) but differ from the initial preliminary values of Keye and colleagues[14] (50 to 80 pg/ml). However, the latter investigators used unextracted plasma and some nonspecificity may have resulted in a higher value.

What is surprising is our inability to detect circulating levels in postmenopausal women in the face of elevation in patients with secondary amenorrhea and midcycle peaks. Similarly, bioassayable GnRH could be detected by Seyler and Reichlin[15] in only seven of 36 samples from postmenopausal women; yet, elevated levels were found by the same investigator in women in midcycle, estrogen-treated men, and long-term castrated patients[16] with the same assay.* Since pituitary gonadotropins are secreted episodically with a periodicity of one and one-half to two hours.[17] peak to peak, presumably the result of GnRH bursts,[18] it is possible that random samples would not detect elevated values of the releasing hormone because of the rapid disappearance rate. It is to be stressed that GnRH with a $T_{\frac{1}{2}}$ of two to five minutes can cause secretion of LH and FSH with significant prolonged disappearance rates ($T_{\frac{1}{2}}$ of at least 30 and 90 minutes[19]) so that the effect of small amounts of GnRH can be amplified many times at the pituitary level. Thus, the periodicity of GnRH may be significantly less than the one and one-half to two hours of gonadotropin secretion. Indeed, the initial data of Seyler and Reichlin[20] indicate that with sequential testing during one day, bioassayable GnRH is not necessarily associated with peak LH bursts. We could not detect differences in normal individuals sampled every two hours from 9:00 A.M. to 11:00 P.M. or every ten minutes for a two-hour period.

*Subsequent to submission of our paper, Bolton (J. Endocrinol. 63: 255, 1974) detected no difference in urinary excretion of GnRH between normal men, postmenopausal women, and patients with Klinefelter's syndrome, which further substantiates our results.

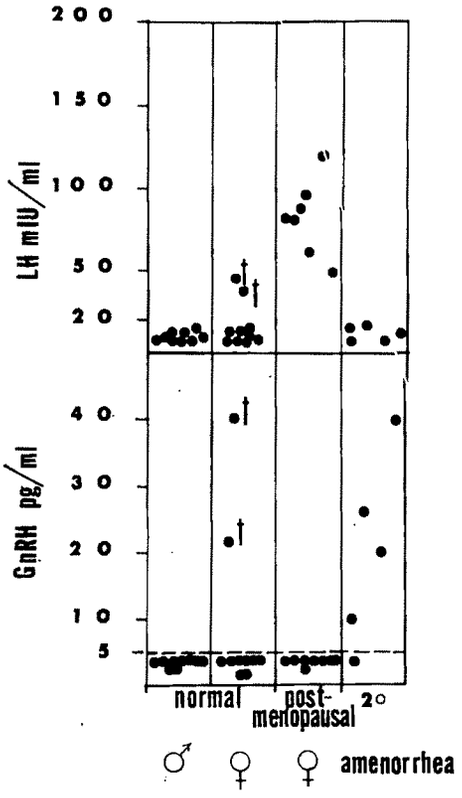


Fig. 7. GnRH and LH values in normal men, women during the follicular, luteal, and midcycle (†), postmenopausal women, and patients with secondary amenorrhea. The dotted line at 5 pg. per milliliter represents the limit of detectability.

The presence of significant random elevations of GnRH in four of five consecutive patients with secondary amenorrhea unassociated with previous medication, weight loss, anorexia nervosa, thyrotoxicosis, or diabetes mellitus stands in significant contrast to the low unmeasurable levels of post-menopausal women. It is clear that if one assumes a purely random oscillation or intermittent periodic bursts of GnRH it would be at best equally unlikely to measure elevated hormone levels in those individuals with secondary amenorrhea. Since the elevations were, indeed, noted on random blood specimens in each case, we feel that our patient data are not explainable simply by the vagaries of random measurement.

It is well known that low-dose estrogen can sensitize the pituitary to exogenous GnRH.[21] The absence of measurable GnRH in postmenopausal patients in the face of markedly elevated gonadotropins would point to pituitary sensitivity as a significant modulating if not controlling factor to the secretion of gonadotropins. However, our estrogen-deficient patients with secondary amenorrhea had elevated GnRH with associated normal LH levels: (normal mean 12.6, range 8.1 to 16.0; secondary amenorrhea mean 14.1, range 10.5 to 19.0, no significant difference). Individuals with secondary amenorrhea and low LH have been reported to have a hypothalamic defect[22] since exogenous GnRH stimulation gives significant pituitary stimulation in the face of low basal and absent episodic secretion of LH. However, our patients did not fall into those clinical states usually associated with hypothalamic amenorrhea such as, psychogenic amenorrhea, acute weight loss, postpill amenorrhea, or anorexia nervosa, nor did they have low basal gonadotropins. Further studies defining the hypothalamic-pituitary axis such as pituitary response and feedback control of GnRH in these patients must be done to define the possible cause of elevated GnRH. These data on normal men and cycling women and patients with secondary amenorrhea provide a point for further evaluation of the hypothalamic pituitary axis in man and with more extensive studies can result in important biological information on the control of secretion of gonadotropins in health and disease.

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Incidence of cervical cancer in perimenopausal and postmenopausal women detected by Papanicolaou smears

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This paper concerns itself with the prevalence of abnormal Papanicolaou smears in women 40 years of age or older. They are considered in two groups, i.e., perimenopausal (40 through 49) and postmenopausal (50 or more). In the perimenopausal group, the incidence of abnormal smears was higher than in a central population of women of all age groups. Abnormal smears were less than half as common in the postmenopausal group. Invasive cancer was four times greater in the perimenopausal group and two times greater in the postmenopausal group.

IT IS THE PURPOSE of this paper to present statistical data concerning abnormal cervical cytologic findings in two limited samples of individuals in the perimenopausal and postmenopausal age groups.

The Papanicolaou smear of the uterine cervix is the modern woman's first defense against death from cancer of this organ. Before its advent, deaths in the United States from cancer of the breast and cervical malignancy were virtually equal. At this time, death from cervical cancer has been reduced to two fifths the rate of death from breast cancer, because of the widespread use and acceptance of the Papanicolaou smear. Procope[1] points out that of 1,085 postmenopausal women who presented with postmenopausal bleeding, 28 per cent were of malignant etiology, one half of these being due to cervical cancer.

Material and methods

In September, 1966, an ongoing cervical cancer detection program was initiated by the St. Luke's Foundation at St. Luke's Hospital of Kansas City. The specimens are obtained through obstetric and gynecology clinics associated with the residency training program at St. Luke's Hospital and the Kansas City, Missouri, Family Planning Agency. The data collection form is portrayed in Fig. 1 and is designed to supply not only pertinent information believed to be important concerning etiologic theories of carcinoma of the cervix but also adequate personal data to allow the social work staff to seek out patients with abnormal smears for appropriate follow-up.

Single slides are prepared for each patient from samples of the squamocolumnar junction taken with a wooden spatula. These are preserved with a fixative spray. All smears are screened by cytotechnologists, with abnormal smears being categorized by a pathologist. All abnormal smears are reviewed by the Department of Obstetrics and Gynecology, and appropriate further diagnostic procedures are decided upon. The staining of the smears is by the standard Papanicolaou technique.

The smears are classified in any one of three categories, according to the absence or presence of abnormal cells. These categories are: (1) negative, meaning no abnormal cells were seen; (2) positive, meaning abnormal cells were seen; and (3) suspicious, meaning no frankly abnormal cells were seen, but all cells were not perfectly normal.

The two patient populations of particular interest in this paper are: (1) the perimenopausal group, which are defined as those women 40 through 49 years old;

From St. Luke's Hospital.

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ST. LUKE'S FOUNDATION CERVICAL CANCER PROJECT

CERVICAL PAP SMEAR REPORT FORM

(1) _____

(10) _____ DATE

(16) _____ LAST NAME _____ FIRST (40) _____ DATE OF BIRTH

(46) ☐ White ☐ Black ☐ Other RACE

_____ ADDRESS _____ HUSBAND'S NAME

_____ HOME PHONE NO.

_____ PLACE OF EMPLOYMENT _____ PHONE NO. AT WORK

(47) _____ SOCIAL SECURITY NO.

_____ NAME OF A RELATIVE OR FRIEND

_____ ADDRESS

_____ PHONE

HISTORY

(56) ☐ Yes
Previous Pap Test ☐ No When _____ Where _____ Results _____

(57) ☐ Yes
Are You Pregnant Now ☐ No
Last Menstrual Period _____ ☐ Regular ☐ Irregular

(58)
How Many Times Have You Been Pregnant _____

(59)
Number of Pregnancies Carried 5 Months or Longer _____

(60) ☐ Yes
Was a Baby Delivered in Last 6 Weeks ☐ No

(61)
Birth Control Method _____ If Pills, What Name _____ Date Started _____

(62)
Age First Experienced Intercourse: Under 15____ 15-19____ 20-24____ 25 & over____

(63)
Age at Delivery of First Child: Under 15____ 15-19____ 20-24____ 25 & over____

COMMENTS:

CLINIC_____ CASE_____

_____MD

CLASS

I ☐
II ☐
III ☐
IV ☐
V ☐

REPORT

(64) FINDINGS					
Negative for Cancer	SUSPICIOUS	POSITIVE	UNSATISFACTORY	TRICH.	INFLAM.

REMARKS:
(68)
(69)

_____MD

Fig. 1. St. Luke's Foundation cancer project: cervical Papanicolaou smear report form.

Table I. Papanicolaou smear results

	Age group					
	40-49		*50 and over*		*All smears*	
Initial smears	1,164		809		38,163	
Suspicious smears per 1,000	32	(37)	15	(12)	22	(804)
Positive smears per 1,000	13	(15)	7.4	(6)	6.5	(249)
Repeat smears	1,928		534		30,275	
Suspicious smears per 1,000	14	(27)	7.5	(4)	13	(404)
Positive smears per 1,000	3	(6)	0	(0)	3	(90)
All smears	3,092		1,343		68,438	
Suspicious smears per 1,000	20.7	(64)	11.9	(16)	18.2	(1,244)
Positive smears per 1,000	6.8	(21)	4.5	(6)	5.0	(339)

and (2) the postmenopausal group, which are defined as those women 50 years of age and older.

Results

In order to assess the adequacy of the samplings, a distinction has been made between initial smears and subsequent smears from the same individual. The subsequent smears have been designated as "repeat" and should reflect newly developing lesions as well as those that escaped detection on the initial examination. Table I reflects these results as suspicious and positive smears per thousand examinations in the age groups outlined above, also contrasts the initial and repeat examinations, and gives a summation of all smears obtained.

Table II shows the tissue diagnoses obtained on further evaluation of those individuals with suspicious and positive smears. The designation "no follow-up required" deserves some explanation. This category pertains to those smears in which the presence of parabasilar cells precluded their designation as negative smears. This situation is compatible with inadequately estrogenized epithelium, and these 49 smears "cleared" after exogenous estrogen administration. Those individuals whose smears were "cleared" in this manner were not subjected to further diagnostic procedures.

Table III reveals the incidence of cervical neoplasia in the various age groups per thousand smears.

Table II. Tissue diagnoses of abnormal smears

	Age group	
	40-49	*50 and over*
Suspicious		
Invasive	1	1
Carcinoma in situ	4	1
Dysplasia	12	1
Nonmalignant	5	0
NFR*	37	12
Pending	5	1
Total smears	65	16
Positive		
Invasive	1	4
Carcinoma in situ	16	2
Dysplasia	2	0
Nonmalignant	2	0
Total smears	21	6

*Follow-up required (see text for explanation).

Table III. Incidence of cervical neoplasia (expressed as cases per thousand smears)

	Invasive carcinoma	*Carcinoma in situ*	*Dysplasia*
Through 39 years	0.077 (5)	4.9 (321)	4.9 (301)
40 through 49 years	0.65 (2)	6.5 (20)	4.5 (14)
50 years and over	3.7 (5)	2.2 (3)	0.74 (1)
All smears	0.18 (12)	5.0 (344)	4.6 (316)

Comment

The results indicate that the perimenopausal age group demonstrates a slightly higher incidence of abnormal smears than the total population, whether they are suspicious or positive. The postmenopausal age group displays an incidence of positive smears about equal to that in the total population; however, the incidence of suspicious smears appears to be reduced. The apparent reduction of incidence in all cases when the initial and repeat screenings are compared may indicate to some degree the efficacy of the technique employed.

Of note in Table II is the wide variety of tissue diagnoses in the suspicious group, while the positive group consists mainly of carcinoma in situ and invasive carcinoma.

An attempt is made in Table III to determine the incidence of the various stages of neoplasia on the basis of the smears performed. Invasive carcinoma was seen in the postmenopausal group 21 times as often as in the total population, while the perimenopausal group demonstrated invasive carcinoma four times as often as the total population. Carcinoma in situ in the perimenopausal group was similar to that in the total population; however, this manifestation was present

less than half as often in the postmenopausal group. The occurence of dysplasia was again similar in the perimenopausal group and the total population, with the postmenopausal group showing dysplasia one sixth as often.

In a computerized study of 137,877 smears, von Hamm and Nikolewski[2] accumulated 18,064 smears from women in the perimenopausal age group. Of these, 60 were suspicious (0.37 per cent) and seven were positive (0.04 per cent). In the postmenopausal age group, 5,193 smears were accumulated with thirteen suspicious smears (0.25 per cent) and seven positive smears (0.13 per cent). Their entire study yielded 306 suspicious smears (0.27 per cent) and 27 positive smears (0.02 per cent). The frequency of abnormal smears in their study is of a much lower magnitude than that presented here. The descrepancy is probably due to the fact that their study population consists of spouses of armed services personnal, while ours is an indigent population. Indigent populations are commonly thought to be at greater risk to develop cervical neoplasia.

Conclusions

The data presented appear to justify the following statements when comparisons are made with the total population: (1) The perimenopausal years hold a slightly greater risk for development of an abnormal smear; (2) the perimenopausal years hold a distinctly greater risk of invasive carcinoma (fourfold); (3) the postmenopausal years hold less risk for development of an abnormal smear; (4) the postmenopausal years hold a decreased chance of carcinoma in situ and a much reduced incidence of dysplasia; (5) the postmenopausal years hold a much greater risk of invasive carcinoma (twenty-one–fold).

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The interpretation and significance of the fractional postcoital test

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This investigation was designed to objectively determine the clinical usefulness of the fractional postcoital test. Forty-three normal subjects had midcycle cervical mucus collection at various times after insemination. There was a significant correlation between the number of motile sperm at the internal os level and the total sperm count within the cervical mucus. The median internal os count was 15 motile sperm per high-power field with a lower 95 per cent confidence limit of five motile sperm per high-power field. Therefore, the fractional postcoital test is useful clinically as it is a physiologic indication of sperm transport in cervical mucus.

THE CLINICAL postcoital evaluation of midcycle cervical mucus of infertile couples is widely practiced. Reasons for the frequent use of this test are: (1) the cervical mucus is the only readily obtainable secretion from the female genital tract; (2) it is only by examining the cervical mucus for spermatozoa that any direct information about the adequacy of insemination can be obtained; and (3) this examination provides information as to how the sperm are interacting with the female secretions.

Sobrero and MacLeod[1] have demonstrated the rapidity of sperm transport to the cervix. In their subjects spermatozoa were found to be present in the cervical mucus as soon as the observer could make his first examination (90 seconds) following normal coitus. It has also been shown that, providing large numbers of actively motile sperm are found in the cervical mucus, the semen analysis of the husband usually is normal.[2] This highly positive degree of correlation between the sperm quality in the cervical mucus and in the ejaculated semen makes it possible to utilize the postcoital test (PCT) as an adequate screening method of sperm penetration in the diagnostic survey of infertile couples. When no sperm or only spermatozoa of poor quality are present in the cervical mucus, however, no definite conclusions can be drawn as to the quality of semen.

The interpretation of what constitutes a positive PCT varies among different investigators. The range of a normal test in reference to the number of sperm varies from one motile sperm per high-power field to 20 or more.[3–5] Davajan and Kunitake[6] emphasized the importance of finding motile sperm in cervical mucus removed from the level of the internal cervical os. They concluded that the number of sperm at the level of the internal os is a more rational test than the number of sperm at the external os as originally described by Sims[7] and Huhner.[8] Davajan and colleagues[9] have attempted to standardize this evaluation by means of a fractional PCT. By comparing fertile and infertile women they noted that in the former the number of sperm per high-power field in cervical mucus ranges from 7 to 15 at the internal os level (2.5 cm.). These investigators have stated that the presence of five or more highly active sperm per high-power field at the internal os level constitutes a normal PCT.

Since the fractional PCT represents a very practical method for evaluating one possible etiology of infertility, a study was undertaken to further characterize and quantitate the fractional PCT. This report represents an extension of preliminary data concerning sperm transport in the human female reproductive tract at

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midcycle[10] which has been presented in detail in a doctoral dissertation.[11] A study regarding the optional time for performing the postcoital test has been previously reported.[12]

Materials and methods

The study group consisted of 43 women, with a history of regular menstrual cycles, whose ages ranged from 21 to 42 years. The investigation took place at midcycle and the cervical mucus spinnbarkeit was greater than 8 cm. in all subjects at the time of insemination. All 43 subjects were examined prior to insemination to ensure the absence of extraneous sperm in the cervical mucus.[10, 11] Each of the study cycles was presumed to be ovulatory on the basis of spontaneous menses after an appropriate luteal phase and/or elevated luteal serum progesterone.

Insemination with fresh semen was performed in a group of 38 subjects. The technique of vaginally inseminating via a syringe has been described in detail previously.[10–12] The five patients who were not inseminated were seen at varying times after natural coitus.

From 5 minutes to 48.8 hours after insemination or coitus, the cervical mucus column was removed by the technique of Davajan and Kunitake.[6] The portio vagina of the cervix was carefully cleansed with a cotton swab. Then a sterile polyethylene catheter, 3 mm. in diameter (standard intravenous connecting tubing), was inserted in the endocervical canal without contamination from the vaginal contents. Advancement of the catheter to the level of the internal os was done while gentle suction was applied to the tubing by a syringe. The ratched clamp used to advance the tube was closed, the tubing was removed from the endocervical canal, and any remaining mucus at the tip of the tubing was cut. In this fashion, cervical mucus present at the tip of the tubing represented that at the level of the internal cervical os and that closer to the syringe represented mucus at the level of the external os. The number of sperm and the per cent motility at the level of the internal os was determined by direct microscopic observation of the mucus in the distal 2 mm. of tubing at 400× magnification. The cervical mucus remaining in the tubing was then dissolved in a mucolytic solution.[11]

The assumptions of the analysis of variance could not be satisfied in this investigation since the sperm count at the internal cervical os level was determined on the ordinal scale. Consequently nonparametric (ranking) and graphic data analysis were done.[13, 14] The data presented were analyzed nonparametrically by Kendall rank correlations and graphically by rankit methods.

Table I. Cervical mucus data

Sample No.	Time after Insemination (min.)	Total cervical mucus sperm		Motile sperm/HPF†	
		×10⁵	Rank*	No.	Rank*
1	5	0.31	15.5	10-15	22.0
2	5	0.45	18.0	>20	42.0
3	10	0.27	11.0	10-15	22.0
4	10	0.19	8.5	5-10	12.5
5	15	0.96	25.0	>10	31.5
6	15	1.04	27.5	>10	31.5
7	30	0.80	24.0	>10	31.5
8	30	1.04	27.5	>30	43.0
9	40	0.75	23.0	>10	31.5
10	40	0.74	22.0	10-11	20.0
11	40	0.25	10.0	8-10	17.5
12	49	2.24	32.0	>10	31.5
13	50	0.62	20.0	>10	31.5
14	50	0.31	15.5	10-15	22.0
15	60	1.82	31.0	>10	31.5
16	60	3.90	38.0	>10	31.5
17	60	0.28	12.5	3-5	7.5
18	60	1.60	30.0	5-10	12.5
19	75	9.90	43.0	20-30	41.0
20	75	2.47	33.0	>5	14.0
21	90	0.68	21.0	8-10	17.5
22	90	1.00	26.0	4-5	9.5
23	150	4.00	39.0	>10	31.5
24	150	5.40	41.0	>10	31.5
25	202	5.90	42.0	12-14	40.0
26	245	2.66	35.0	10	19.0
27	245	0.29	14.0	7-10	16.0
28	330	2.97	37.0	>10	31.5
29	390	1.06	29.0	>10	31.5
30	390	0.08	6.0	>10	31.5
31	480	0.34	17.0	>10	31.5
32	480	0.19	8.5	2-3	6.0
33	600	2.77	36.0	>10	31.5
34	740	2.49	34.0	3-5	7.5
35	750	0.28	12.5	>10	31.5
36	1200	0.10	7.0	4-5	9.5
37	1440	4.30	40.0	6-10	15.0
38	1440	0.59	19.0	5	11.0
39	1440	0.07	5.0	1	4.5
40	1665	0.002	3	1	4.5
41	2640	0.00	1.5	0	2
42	2875	0.00	1.5	0	2
43	2929	0.03	4	0	2

*Ranking data for nonparametric analysis.
†Internal cervical os level.

Results

The sperm count of the internal cervical os level (distal 2 mm. of the polyethylene tubing) and the total number of cervical mucus sperm remaining in the polyethylene tubing at various times after insemination are presented in Table I. The ranking scale of each sperm count is also listed in Table I. Note that on an ordinal scale of measurement, > 30 sperm per high-power field has a higher rank than > 10 sperm per high-power field.[13] It has been reported previously that the cervical mucus sperm count of the subjects

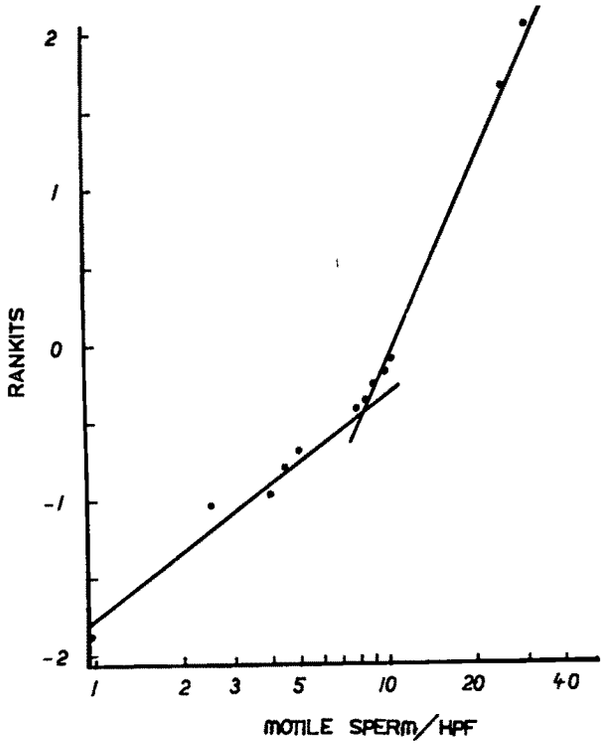


Fig. 1. Number of motile sperm per high-power field at the internal cervical os. Linear transform method (rankits) reveals two different populations. The first group is characterized by a median collection time of 1,440 minutes after insemination; the second group is 60 minutes (N = 43).

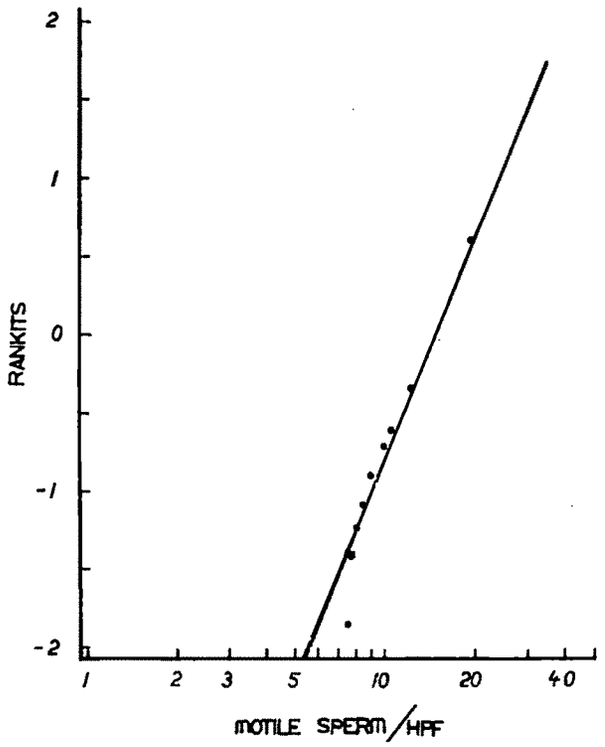


Fig. 2. Number of motile sperm per high-power field at the internal cervical os in group 2 (N = 32).

after natural coitus corresponds to that of those inseminated via the syringe.[12]

With a nonparametric measure of correlation,[13] there is a significant correlation between the internal cervical os sperm count and the total cervical mucus count. The Kendall rank correlation (τ) coefficient is 0.420, which yields a z value of 3.97 ($p < 0.001$). As is evident from Table I, however, three variables are present: internal cervical os sperm count, cervical mucus sperm count, and time. Therefore, the data were further analyzed by the Kendall partial rank correlation ($\tau xy.z$). This partial correlation determined whether a correlation existed between the internal cervical os sperm count and the cervical mucus count with the third variable, time, kept constant. When this condition was satisfied, the correlation was still significant. The Kendall partial rank correlation ($\tau xy.z$) is 0.406 instead of 0.420, which still generates a significant z value of 3.83 ($p < 0.001$).

The internal cervical os sperm counts were then graphically analyzed by a linear transform method. Rankit analysis revealed two different populations for the sperm counts at the internal os (Fig. 1). The two populations differed in the time of collection after insemination. The median collection time after insemination in the first group (11 of the 43 samples) was 1,440 minutes while the second group was 60 minutes. The second group is further characterized by the graphic analysis depicted in Fig. 2. Rankit analysis reveals that the median sperm count at the internal os level is 15 motile sperm per high-power field. The lower 95 per cent confidence limits (2 S.D.) is five to six motile sperm per high-power field. Comparable graphic analysis of the total cervical mucus sperm counts revealed similar populations and a median value of 7.8×10^4 sperm.

Comment

The present investigation was designed to measure sperm and cervical mucus interaction in order to further characterize and quantitate the fractional PCT. Davajan and co-workers[6, 9] demonstrated a difference between the internal cervical os sperm count obtained from fertile and infertile patients with no previously determined etiologic factor. If their statement is true that the number of sperm at the level of the internal cervical os is a reflection of the cervical factors in infertility, then the sperm count at the level of the internal cervical os should correlate with the total sperm count of cervical mucus in normal subjects

irrespective of the time of collection after insemination or coitus. The results of this study reveal a definite correlation between the motile sperm count at the internal cervical os and the total cervical mucus count ($p < 0.001$). This positive correlation confirms the clinical findings of Davajan that the fractional method of testing postcoital cervical mucus is a true reflection of sperm transport in cervical mucus. Consequently, it can be used as an accurate and practical means to diagnose the cervical cause of infertility.

In regard to what constitutes a normal PCT, Davajan originally stated that five or more highly active sperm per high-power field at the internal os level of cervical mucus was normal. Recently, however, he[15] stated that two or more motile sperm per high-power field at the internal os could be normal, since some of his infertility patients have become pregnant with that count. As noted in Fig. 2, the median sperm count at the internal os level is 15 motile sperm per high-power field; however, the 95 per cent lower confidence limit is five motile sperm per high-power field. Therefore, statistically, the lower limit of a normal fractional PCT is five motile sperm per high-power field with 95 per cent confidence. Occasionally fewer than five motile sperm per high-power field will be found in the cycle of conception, but this finding should occur less than 5 per cent of the time.

It has been previously reported that the postcoital examination should be optimally performed within 2½ hours after coitus.[12] This is evident since there is a significant statistical increase in the ratio number of motile inseminated sperm to the number of sperm within the cervical mucus after 150 minutes. Therefore the fractional postcoital test performed within 150 minutes after coitus is useful clinically as it is a physiologic indication of sperm transport in cervical mucus. Provided more than five motile sperm per high-power field are found at the internal os level, the couple has normal sperm transport in the cervical canal. If fewer than 5 motile sperm per high-power field are seen, then the test is abnormal and may account for the infertility. Thus the fractional PCT provides a more precise and easily defined method of determining sperm transport in cervical mucus than the original test described by Sims and modified by Huhner.

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Periovulatory changes in ovarian temperature in ewes

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Thermistors were implanted in intact ewes, and ovarian and aorta temperatures were recorded for 72 hours following the removal of a progesterone implant intended to induce estrus. Serum levels of luteinizing hormone (LH) were measured hourly over a 10 hour period beginning at the onset of estrus. Ovaries were examined by laparotomy at the end of each experiment. Estrus behavior, LH surge, and a fresh corpus luteum were all found to be present in four, and absent in two, of a total of six experiments. To remove variations in basal body temperature, ovarian temperatures were measured relative to aorta temperature at all times. In the nonovulating ewes, both mean ovarian temperatures varied at random, whereas in the ovulating ewes they decreased by approximately 0.15° C. to reach a minimum 1 hour before the time of maximum rate of increase in LH. The ovulating ovaries differed from the contralateral ovaries (where ovulation did not occur) in two respects: the mean temperature in the ovulating ovaries was lower by 0.02 to 0.10° C. at all times, and its preovulatory decrease began several hours earlier. When taken in conjunction with published data on secretion and on the local and systemic effect of estradiol, these findings are consistent with the hypothesis that ovarian estradiol induces an increase in ovarian blood flow. A heat-sink effect of the uterine mass due to its proximity to the ovaries may also be involved.

HORMONE-DEPENDENT temperature changes known to occur during the ovarian cycle in mammals are of two types which differ in cause, effect, and mechanism. Through central metabolic action, progesterone from the corpus luteum induces the familiar postovulatory rise in basal body temperature which has been extensively studied and widely used for the retrospective detection of ovulation in women.[1] When secreted by the growing follicle at an increasing rate, peaking in sheep 0 to 8 hours before the onset of estrus,[2] estradiol increases blood flow in the reproductive tract, and this change in blood flow, through the mechanism of perfusion and heat removal, induces local temperature changes in the tissues involved.[3-5]

Local ovarian hyperemia is a well-known early effect of gonadotropins on the rodent ovary, and has been used as an end-point in the bioassay of these hormones,[6] but the local temperature change likely to accompany the increase in blood flow—whether induced by the administration of gonadotropins, or physiologically at certain phases of the ovarian cycle—does not seem to have been established. When ovarian temperature was telemetered intermittently over several months in rhesus monkeys, the readings fluctuated by as much as 1.7° C. in any 24 hour period, there was some parallelism with rectal temperature changes, and the investigators concluded that continuous circadian temperature rhythms would be needed before further interpretation.[7]

Reproductive tract temperatures in sheep exceed aorta or basal body temperature by no more than 0.2 to 0.4° C.,[3] and basal body temperature in rhesus monkeys may vary throughout a day by as much as 2° C.[8] Although these data refer to different species, they do

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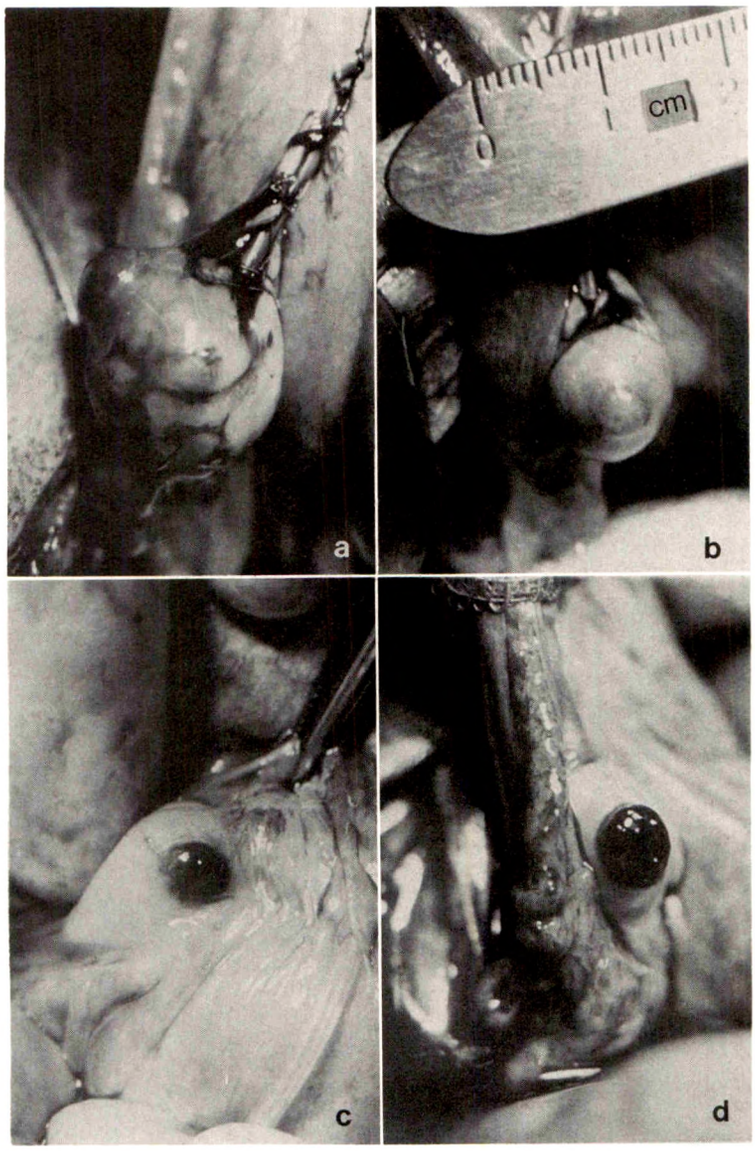


Fig. 1. Thermistor implants and corpora lutea in the ovaries of Ewe No. 39C. Right *(a)* and left *(b)* ovary with old corpus luteum on the day of implantation. Left ovary with fresh corpus luteum at the end of Exp. 2, 15 days after implantation *(c)*, and at the end of Exp. 3, 36 days after implantation *(d)*; the thermistor leads are covered by adhesions.

indicate an order of magnitude of the variation to be expected in any one animal. Attempts to detect thermal shifts due to cyclic ovarian function are likely to fail unless the variation in basal body temperature is taken into account.

The design of the present investigation was based on these data and considerations. Its objective was to record ovary temperature bilaterally along with aorta temperature in ewes around the time of ovulation, in order to ascertain whether a cycle-dependent feature in ovarian temperature could be detected when the temperature component dependent on basal metabolism was removed. The significance of such studies lies in the possibility that they may lead to the development of electronic devices for the detection or prediction of ovulation.

Material and methods

Aorta and ovarian tissue temperatures were measured in ewes by means of implanted glass bead thermistors (diameter, 1 to 2 mm.) with hard-wire connections to bridge amplifier circuits, and continuous recording on a strip chart. The system was calibrated with a water-bath over the interval of 34 to

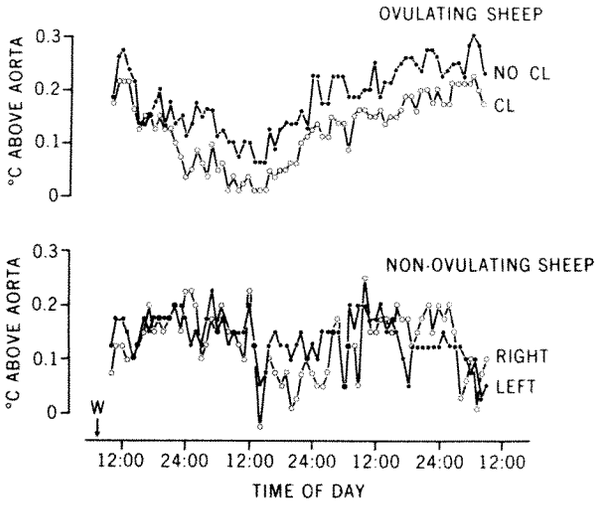


Fig. 2. Ovarian temperature in ewes measured relative to basal body temperature (aorta) after withdrawal of progesterone *(W)* to induce estrus. Plotted points are averages over ovulating ovaries (*CL*, fresh corpus luteum found at laparotomy) and over the quiescent contralateral ovaries *(NO CL)* in four experiments with occurrence of ovulation and over right and left ovaries in two anovulatory experiments.

42° C. Drifts over 15 days of individual bridge amplifier outputs were between 0.005 and 0.080° C.

Five Dorset ewes, 2 to 5 years old and with regular behavioral estrus patterns, were used in six experiments, i.e., one animal (Ewe No. 39C) was used twice (Exps. 2 and 3). The animals were kept at 22° C in individual pens 3 M. square, with lighting adjusted weekly to match the duration of actual daylight.

For the control of estrus, a silicone rubber implant impregnated with progesterone was placed subcutaneously.[9] One week later, surgery was carried out to insert one thermistor probe into the medulla of each ovary, after a pathway was prepared with a 16 gauge hypodermic needle; thermistor leads were sutured to the ovary capsule (Fig. 1, *a* and *b*). A third thermistor was sewn to the exterior ventral wall of the aorta near the uterine-artery branching. Leads were taken through the skin at the flank, and connection to sensor bridges was made through coiled cables permitting complete freedom of movement within the pen.

One week after surgery, the progesterone implant was withdrawn at 9:00 A.M. to induce estrus and recording of three temperature variables (aorta, left ovary, and difference left-right ovary) was begun and continued for 72 hours. Estrus behavior was tested with two rams (one vasectomized, the other intact) at 1 hour intervals beginning 24 hours after progesterone withdrawal and ending either at the onset of estrus or 48 hours after progesterone withdrawal. Jugular blood specimens for assay of luteinizing hormone (LH) were drawn at 1 hour intervals over a 10 hour period beginning at the onset of estrus.

Finally, both ovaries were photographed and examined for the presence of fresh corpora lutea, at a laparotomy carried out either 36 to 60 hours after the onset of estrus or—in ewes not coming into estrus—72 hours after progesterone withdrawal.

For numerical evaluation, average values over successive 1 hour intervals were read from the continuous temperature tracings, and the measurements were expressed for each ovary and hour as degrees Celsius above the corresponding aorta temperature.

The concentration of LH was determined in 200 μl samples of serum according to a double-antibody radioimmunoassay method.[10] The tracer was a purified preparation of ovine LH (Reichert's LER-1056-C2) iodinated with iodine-125. Rabbit antiserum to ovine LH (Niswender's No. 573) was used at a final dilution of 1:800000. The second antibody (antiserum to rabbit γ-globulin) was prepared in our laboratory. A preparation of ovine LH (NIH-S-15) was used as standard, and results were reported as weight equivalents of this standard. Under the conditions used, the detection limit of the assay was 0.5 ng. per milliliter.

Results

Three indicators of imminent or recent ovulation—namely, estrus behavior, LH surge, and a single fresh corpus luteum in one ovary—were all found to be present in four and absent in the other two of the six experiments. Some ovarian adhesions were usually found at laparotomy but there was never any ambiguity in corpus luteum identification (Fig. 1, *c* and *d*). Ovaries which did not contain a fresh corpus luteum were invariably small and unstimulated in appearance, much like those seen outside the estrus season. It is noteworthy that, in this series, no ewe had fresh corpora lutea in both ovaries at the same time. One ewe, which we used twice (Fig. 1) and had scheduled for a third experiment, was found to have become pregnant during the second experiment and had a normal lamb 149 days after estrus.

Temperature tracings showed random variations of ±0.1° C. or less within most 1 hour intervals. Aorta temperature varied between 37.8 and 39.8° C. with a circadian rhythm.

In all ewes, both ovary temperatures were greater than aorta temperature at all times.

Average ovarian temperatures above aorta were calculated in several ways. For the purpose of compar-

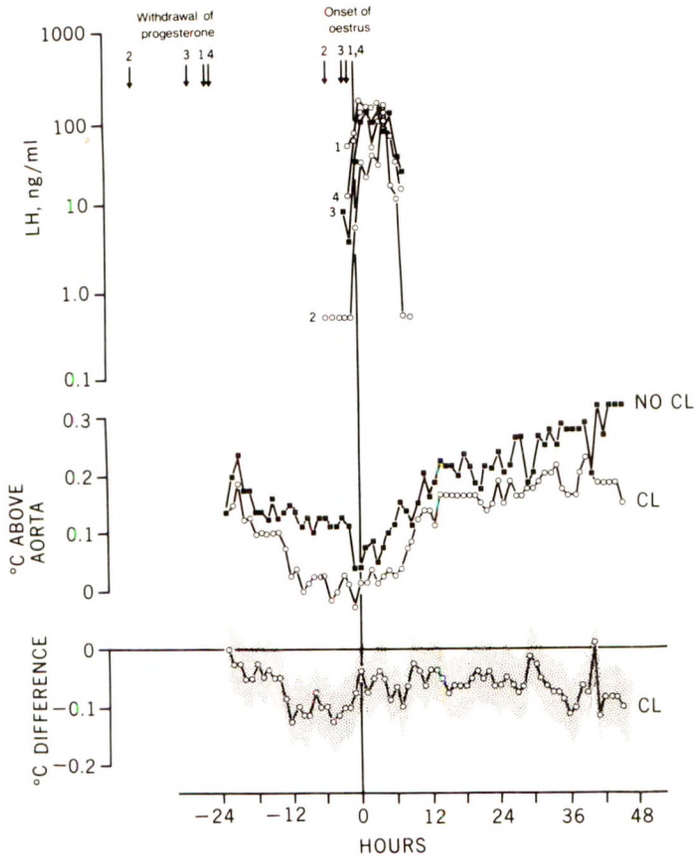


Fig. 3. Ovarian temperature and serum LH level in ovulating sheep (same experiments as in Fig. 2). LH levels, time of onset of estrus, and time of progesterone withdrawal are shown individually for each of the four experiments (1 to 4). The time scale is in hours before and after the time of maximum rate of LH increase indicated by the vertical line at time 0, and the plotted temperature averages over ovulating *(CL)* and quiescent *(NO CL)* ovaries were calculated with respect to this time scale. The bottom curve represents the mean temperature of ovulating ovaries relative to that of the contralateral quiescent ovary put equal to zero; the shaded area extends to 1 S.E. above and below the mean of these differences (*CL* minus *NO CL*).

ing the ovulating sheep (four experiments) with the nonovulating sheep (two experiments), the curves were aligned with respect to the time of progesterone withdrawal. The resulting hourly mean values over the ovulating and the nonovulating ovaries in the ovulating ewes, and over the right and the left ovaries in the anovulatory animals, are shown in Fig. 2. In the nonovulating ewes, the two ovarian temperatures varied independently and at random around a mean near 0.14° C. above aorta. In the ovulating ewes, both ovarian temperatures began to decrease shortly after progesterone withdrawal and continued to approach aorta temperature until reaching a minimum about 30 hours after progesterone withdrawal. They then rose again at a similar rate to the preovulatory level. From about 12 hours after progesterone withdrawal until the end of the recording 60 hours later, the mean temperature of the ovulating ovaries (i.e., those where the one fresh corpus luteum was found at laparotomy) was lower at all times than that of the contralateral ovaries.

For the purpose of examining further this difference between ovulating and nonovulating ovaries in the same animal, the hourly mean temperature values plotted in Fig. 3 were calculated after alignment of the individual curves with respect to the maximum rate of LH increase. The mean temperature in the ovulating ovaries was again consistently lower than in those not ovulating. The lowest mean temperature occurred 1 hour before the reference point in both ovaries, but the anovulatory ovaries lagged about 12 hours behind the ovulating ovaries in the beginning of the decrease.

When we examined alignment of the temperature curves with respect to the time of progesterone

withdrawal (as for Fig. 2), onset of estrus, or LH peak, none of these reference points revealed different or more clearly defined correlations. The relation between the four reference points in time is shown in Fig. 3 for each of the four experiments.

Comment

Estrus, LH surge, ovulation, and corpus luteum formation all occurred in five out of seven experiments (including a seventh experiment without temperature recording, not otherwise referred to in this report), and conception followed by birth of a normal lamb occurred in one of these; this attests the innocuous nature of the sensor implants which, on this evidence, did not interfere with normal reproductive processes.

The experiments generated their own controls in two respects which, though necessarily unplanned, contributed significantly to the interpretation of the findings. First, removal of the progesterone implant failed to induce an estrus cycle in two animals; this permitted comparison between estrous and anestrous ewes. Second, it so happened that, in all four ovulatory cycles, ovulation occurred in only one ovary; this permitted comparison between ovulating and nonovulating ovaries within the same animal.

Without the differential design which eliminated the variation in basal body temperature, it would not have been possible to demonstrate the suspected hormone-related variation in ovarian temperature, since this was of the same magnitude as the individual hourly variations.

According to current concepts, basal gonadotropin secretion is involved in initiating follicle growth and estradiol secretion after withdrawal of the progesterone block. Our findings suggest, in addition, the hypothesis that ovarian estradiol induces an increase in blood flow not only in uterus and vagina but also in the ovary itself. If the observed decrease in ovarian temperature is attributed to convection, i.e., to heat removal through increased blood flow, both the smaller magnitude and the later onset of the temperature decrease in the anovulatory (as compared with the ovulating) ovaries could well be explained by the difference in estradiol concentration between the secreting and the contralateral quiescent ovary. In fact, published data show that in the ewe estradiol is secreted mainly from the ovary destined to have follicles which mature and rupture,[11] and that response to estradiol in contralateral tissue is diminished by circulatory dilution.[12, 13]

Heat conduction through physical contact of the ovaries with the uterus may also be a factor contributing to the ovarian temperature changes, since uterine blood flow and temperature are known to vary with estrogen levels,[3] and the proximity of the uterine mass to the ovaries may well produce a heat-sink effect.

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Pregnancy with bilateral ruptured benign cystic teratomas

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A case of intra-abdominal rupture of bilateral cystic teratomas in the first trimester of pregnancy is reported. Review of the literature revealed 22 cases of intraperitoneal rupture of benign cystic teratoma (1908 to 1971). In all of these cases, only one ovary was involved, and the period of gestation was after the first trimester.

A CASE OF intra-abdominal rupture of bilateral cystic teratomas in the first trimester of pregnancy is reported.

Case report

An 18-year-old, unmarried, black nulligravida was admitted to the Gynecology Service on February 1, 1974, with a history of left lower quadrant pain, vaginal discharge, six weeks of amenorrhea, and a temperature of 100.2° F. The patient's last menstrual period was on December 12, 1973. Pregnancy test was positive, and ectopic pregnancy was suspected. Culdocentesis was performed, and a small amount of purulent material was obtained. The patient was treated for pelvic inflammatory disease with antibiotics. She was discharged on February 8, 1974, with a diagnosis of pelvic inflammatory disease and possible intrauterine pregnancy.

The patient was seen in the Prenatal Clinic on February 14 and 28, 1974. She had no specific complaints. A pelvic examination on February 28, 1974, revealed a soft, retroverted uterus at eight to ten weeks' gestation.

The patient was admitted to the St. Louis City Hospital through the Emergency Room on March 12, 1974, with a chief complaint of acute lower abdominal pain of two days' duration. Two days prior to this second admission, the patient had developed acute lower abdominal pain, nausea, and vomiting, and a foul-smelling vaginal discharge was noted. She was examined in the Emergency Room and was sent home after being given antibiotics and vaginal suppositories.

On admission, the patient had a temperature of 100.6° F.; pulse, 160 beats per minute; respirations, 36 per minute; blood pressure, 130/80 mm. Hg. The patient seemed to be in acute distress. On auscultation, there was marked tachycardia, and diminished air entry was noted at the lung bases. The abdomen was distended. Rigidity and guarding were present over the entire abdomen. There was marked direct and rebound tenderness, and bowel sounds were absent.

On pelvic examination, there was a foul-smelling discharge in the vagina. The cervix was soft, blue, and closed. Movement of the cervix was very painful for the patient. The uterus was anteverted, soft, and 12 weeks' gestation in size. The posterior cul-de-sac was bulging, and there was marked tenderness. A diagnosis of intrauterine pregnancy with ruptured tuboovarian abscess was considered. Culdocentesis was performed and yielded 7 to 8 c.c. of yellowish green foul-smelling purulent material. Hemoglobin was 13 Gm. per cent, and hematocrit was 40 per cent. White blood count was 20,800 per cubic millimeter with 74 per cent segmented cells, 15 per cent stab cells, 1 per cent juvenile cells, and 10 per cent lymphocytes.

The patient was treated with cephalothin intravenously, 2 Gm. every four hours, and kanamycin, 0.5 Gm. every 12 hours intramuscularly, and prepared for exploratory laparotomy.

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Table I. Laboratory data

	Complete blood count			*Electrolytes*					
Date	*Hgb. (Gm. %)*	*Hct. (%)*	*WBC (/cu. mm.)*	*Sugar (mg. %)*	*BUN (mg. %)*	*Na (mEq./Kg.)*	*K (mEq./Kg.)*	*CO_2 (mEq./Kg.)*	*Cl (mEq./Kg.)*
3/12/74	13	40	20,800	150	8	130	4	27	90
3/13/74	11.9	34.9	21,100	90	7	130	3.5	29	96
3/14/74	11.3	33.2	16,000	90	5	135	4.2	32	96
3/15/74	10.3	29.7	19,900	95	3	131	3.7	33	95
3/16/74	10	31	23,600	91	5	135	3.8	35	91
3/17/74									
3/26/74	9.6	29	19,000						
4/1/74	8.5	27	12,000						

Hgb. = Hemoglobin; Hct. = hematocrit; WBC = white blood cell count; BUN = blood urea nitrogen.

The patient's abdomen was explored through a low midline incision. The abdominal cavity was full of foul-smelling, seropurulent fluid. About 500 to 600 c.c. of fluid was aspirated. The omentum was adherent to the uterus. The uterus was anteverted, 12 weeks' gestation in size, and covered with a film of seropurulent material. Posterior to the uterus, there were two large cystic masses, each about 8 cm. in diameter. As the bowel was being packed, sebaceous material and hair were found floating in the abdomen. A diagnosis of bilateral ruptured infected benign cystic teratomas was made. The right side was explored first, and a ruptured "dermoid cyst" 8 cm. in diameter was removed. Purulent material, hair, and fatty material were pouring out through the ruptured cyst wall. Right salpingo-oophorectomy was performed. The left side was then explored, and hair and fatty material were also seen leaking from the left ovarian cyst. The left adnexal mass was then freed of adhesions and "delivered" out of the cul-de-sac of Douglas. A left salpingo-oophorectomy was performed. The intrauterine pregnancy remained undisturbed although the uterus was irritated. Intramuscular progesterone was given during operation. Two hemovac drains were placed in the cul-de-sac, and the abdomen was closed. The pathologic diagnosis was "bilateral benign cystic teratomas with tuboovarian abscesses."

Over the following three days, the patient continued to have tachycardia of 160 beats per minute, and the temperature ranged from 102 to 103° F., with 30 respirations per minute. A chest x-ray showed bilateral pneumonitis before operation and it became worse afterward.

On the fourth postoperative day, the patient started coughing up large amounts of frothy sputum. She had rales at both bases. Medical consultation was obtained, and a diagnosis of congestive heart failure was made. Digitalis was administered, and 40 mg. of furosemide was given intravenously.

The patient showed some improvement over the next two days. The left lung base continued radiographically to show opacity but the right lung cleared rapidly. On the ninth postoperative day, a mass in the left upper quadrant was detected. A diagnosis of subphrenic abscess was confirmed by the surgical consultant. On March 21, 1974, a left subphrenic abscess was drained. On March 26, 1974, the abdominal wound was opened, and 500 c.c. of pus was drained. The abdominal wound was thereafter irrigated daily with hydrogen peroxide.

The subphrenic abscess continued to drain with low Gomco suction. On April 7, 1974, the drain was removed. The abdominal incision was granulating well. The patient was discharged in good condition on April 9, 1974.

All of the patient's pertinent laboratory data are given in Table I.

Comment

Benign cystic teratomas account for 22 to 48.5 per cent of ovarian neoplasms complicating pregnancy.[1, 2] The frequency of complications is said to be more common during pregnancy. Peterson and associates[1] found a complication rate of 24 per cent during pregnancy as opposed to 19 per cent in the nonpregnant state. The overall incidence of rupture of cystic teratomas is 1.2 per cent, but in pregnancy it has been quoted to be as high as 15.8 per cent.[3]

We have reviewed 21 cases of intraperitoneal

Cultures	Blood gases			
	pH	*O_2 (mm. Hg)*	*CO_2 (mm. Hg)*	*HCO_3 (mEq./Kg.)*
Blood—no growth Peritoneal fluid—no growth Cervix—100% alpha strep.	7.46	36	36.5	23.8
Urine—no growth	7.43	76.5	36.8	23.9
sputum—growth of normal flora	7.47	42.2	40.2	28.7

rupture of cystic teratomas in association with pregnancy and added the present case. The 22 cases are summarized in Table II. These cases were analyzed with regard to the etiology, presenting symptoms, and the maternal and fetal outcome.

The causes of rupture of cystic ovarian masses included torsion (2), infection (4), and trauma (7). In nine of the reported cases, the cause was unknown.

In the majority of cases, torsion of ovarian cysts occurs in the second trimester.[1] On the other hand, rupture appears to be rare at this time with only two cases being reported.[4, 5] Only one of these was due to torsion, the other case of torsion with rupture being in the third trimester. An analysis of the seven cases in which trauma was the precipitating factor revealed that the cysts rupture spontaneously during labor in three patients.[6, 7] Cysts were aspirated during labor and then ruptured in two patients.[6, 8] The cyst ruptured while amniocentesis was being performed in one patient,[9] and coitus was the precipitating cause of the acute episode in one patient.[10]

We could find no reported case of malignant degeneration precipitating rupture during pregnancy.

In the remaining eight cases, no cause was apparent, but, as most of these were diagnosed during the puerperium, trauma during labor may have been the precipitating factor.

After a review of the literature, it is apparent that patients have presented with two different clinical aspects of intraperitoneal rupture.[1, 11, 12] One group of patients presented with the clinical picture of an acute episode of sudden onset, and the diagnosis of ruptured cystic teratoma was made after celiotomy. The other group was of the "chronic," slow, leaking type. The patients were generally seen after the acute episode had passed and presented with vague abdominal pain, distention, and a pelvic mass. They were operated upon weeks or even months after the cysts had ruptured. In general, the acute presentation is the more rare; Kistner[11] found 5 of 22 acute cases, and Peterson and associates[1] reported only 5 of 13. However, of the 22 cases associated with pregnancy, 17 patients presented with the acute abdominal picture and required celiotomy during the acute episode. Celiotomy was delayed for periods of seven weeks to eight months in four patients, and one patient died after fat embolism.

The treatment of choice is surgical removal of the ruptured cyst and investigation of the other ovary by "bivalving" it. Thorough peritoneal lavage should be performed.

Kistner[11] was the first to advocate the regimen of abdominal drainage, intravenous fluids, nasogastric suction, and prophylactic antibiotics. Most authors agree with this management.

Three maternal deaths have been reported. One patient died 13 months following treatment; she had a cerebral vascular accident.[13] Another patient died after refusing surgical treatment; she was "dead on arrival" on the third postpartum day.[6] The third patient died after generalized fat embolism three hours following delivery.[14]

Maternal morbidity is difficult to assess as many authors fail to allude to it. Hospital stay varied from five days to three months in the 22 cases reported. Six cases were complicated by chemical peritonitis and paralytic ileus. Two patients developed a subphrenic abscess and wound infection. It is of some interest that in both of these cases the ruptured cysts were infected. (The two patients of Peterson and associates in whom there was infection were stated to have had a "stormy postoperative course").

Fetal outcome has been almost uniformly good. Thirteen patients had already been delivered before rupture or were delivered shortly after the rupture. Six patients were in the third trimester at the time of rupture. In three of these, the infant was delivered by cesarean section at the time of celiotomy.[9, 10, 15] In two cases, pregnancy was allowed to continue after operation. One patient went into premature labor 24 hours after operation and had a normal delivery.[19] The other patient carried the pregnancy to term. One patient was operated upon eight months after delivery.[16]

Two patients were in the second trimester. One

Table II. Review of reported cases of ruptured benign cystic teratomas associated with pregnancy

No.	*Author*	*Age*	*Gravidity-parity*	*Stage of gestation*	*Time of laparotomy*	*Hospital stay (days)*
1	Randall and associates,[13] 1908	26	–	3 Wk. post partum	27 Days	–
2	Walker,[5] 1912	35	1 = 1	Labor	12 Hr.	–
3	Spencer,[6] 1920	–	–	Labor	1 Mo.	–
4	Spencer[6]	–	–	Labor	–	–
5	Spencer[6]	–	–	3 Days post partum	–	–
6	Van Orman and Mautner,[17] 1951	25	2 = 1	28-30 Wk.	8 Mo.	–
7	Quer and co-workers,[7] 1951	24	–	Post partum	July, 1952	–
8	Kistner,[11] 1952	31	3 = 2	32	2 Hr.	10 Days
9	Bell and associates[4] 1954	28	1 = 0	20	5 Days	18 Days
10 to 13	Peterson and colleagues,[1] 1955	–	Four cases, two infected. All in the immediate postpartum period. Not described			
14	Ranney,[18] 1970	17	1 = 1	Labor	3 Mo.	5 Days
15	Waddell and co-workers,[19] 1961	22	3 = 2	36 Wk.	8 Hr.	–
16	Pritchard,[14] 1965	31	2 = 1	Labor	–	–
17	Schnecke,[8] 1966	30	1 = 0	38 Wk.	12 Hr.	15 Days
18	Boehm and associates,[10] 1968	24	5 = 5	41 Wk.	2 Hr.	12 Days
19	Cox and colleagues,[16] 1971	30	3 = 2	16 Wk.	7 Hr.	8 Days
20	Harris,[9] 1971	20	1 = 1	In labor	70 Hr.	–
21	Lee,[15] 1971	36	3 = 2	32 Wk.	72 Hr.	90 Days
22	Mehra and co-workers, 1974	18	1 = 0	12 Wk.	12 Hr.	28 Days

R. = Right; L. = left; TAH = total abdominal hysterectomy; BSO = bilateral salpingo-oophorectomy; RSO = right salpingo-oophorectomy; LSO = left salpingo-oophorectomy; LSCS = low-segment cesarean section.

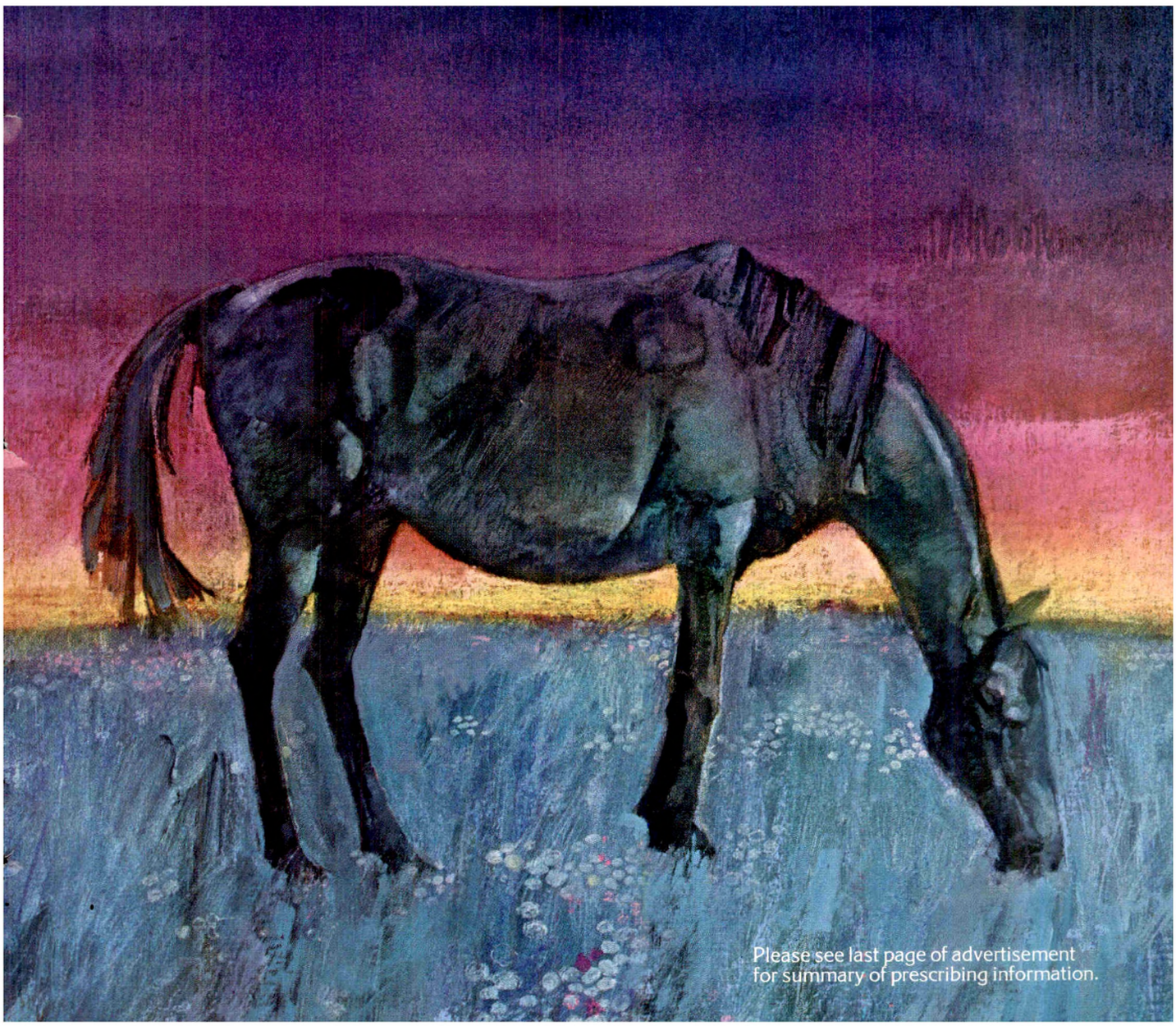
Clinical picture	*Findings at laparotomy*	*Operation*	*Maternal outcome*	*Fetal outcome*
Persistent pain, fever, vaginal discharge	5.5 by 4 cm. R. ovarian dermoid cyst ruptured	–	Died 13 mo. later, cerebral vascular accident	Not stated
Vomiting, abdominal distention—immediately postpartum	3 inch diameter R. ovarian dermoid cyst ruptured	–	Paralytic ileus	Not stated
Ruptured during labor. Removed 1 mo. later	–	–	–	–
Initially tapped, then removed	–	–	–	–
Advised to have laparotomy—refused; collapsed 3 days postpartum	–	–	Dead on arrival ("syncope")	–
Acute episode subsided. Vague abdominal symptoms and mass 6 mo. after delivery	L. ovarian cyst, L. ovarian dermoid. Contents of dermoid in pouch of Douglas	TAH and BSO	–	–
Abdominal pain, vomiting, and distention; initially treated by suction	Biopsy (carcinoma?), L. ruptured, R. dermoid	1. Biopsy 2. RSO	Uneventful	–
"Acute abdomen"	Twisted ruptured L. dermoid	LSO	Paralytic ileus	Uneventful labor and delivery at term
"Acute abdomen"	Ruptured L. dermoid	L. oophorectomy	Chemical peritonitis, threatened abortion	Uneventful delivery at term
Cyst aspirated during labor. refused laparotomy later. Vague symptoms and pelvic mass	Multiple adhesions and granulomas	LSO, cornual resection, adhesionlysis	No complications	Live
"Acute abdomen"	Perforated L. dermoid	L. oophorectomy	Premature labor, 24 hr. postop.	Live
Rigor, hemoptysis, and coma at full dilatation	None	Autopsy, fat embolus from ruptured L. dermoid	Died 3 hr. post partum	Live
Severe dyspareunia, "acute abdomen"	Bilateral dermoid. R. ruptured	LSCS, RSO, L. ovarian cystectomy	No complications	Live
"Acute abdomen" following amniocentesis. Diagnosis: abruptio placentae	L. dermoid ruptured	Cesarean section, TAH and LSO	Chemical peritonitis	Live
"Acute abdomen"	Twisted ruptured L. dermoid	L. oophorectomy	No complications	Live
"Acute abdomen" in early puerperium, 2° arrest of dilation	Ruptured R. dermoid	R. oophorectomy	No complications	Live
"Acute abdomen"	Infected ruptured R. dermoid	Classical cesarean section, RSO, L. salp., Transverse colostomy, perforated sigmoid	1. Subphrenic abscess 2. Wound infection	Live
"Acute abdomen"	Bilateral ruptured dermoid, infected	BSO	1. Subphrenic abscess 2. Wound infection	Abortion

pregnancy progressed normally.[4] There was threatened abortion in the second case, but eventually pregnancy was terminated at term.[5]

Our case is apparently the first reported where bilateral cystic teratomas ruptured in the first trimester. Despite exogenous progesterone, a combination of severe generalized peritonitis and manipulation during operation probably caused abortion.

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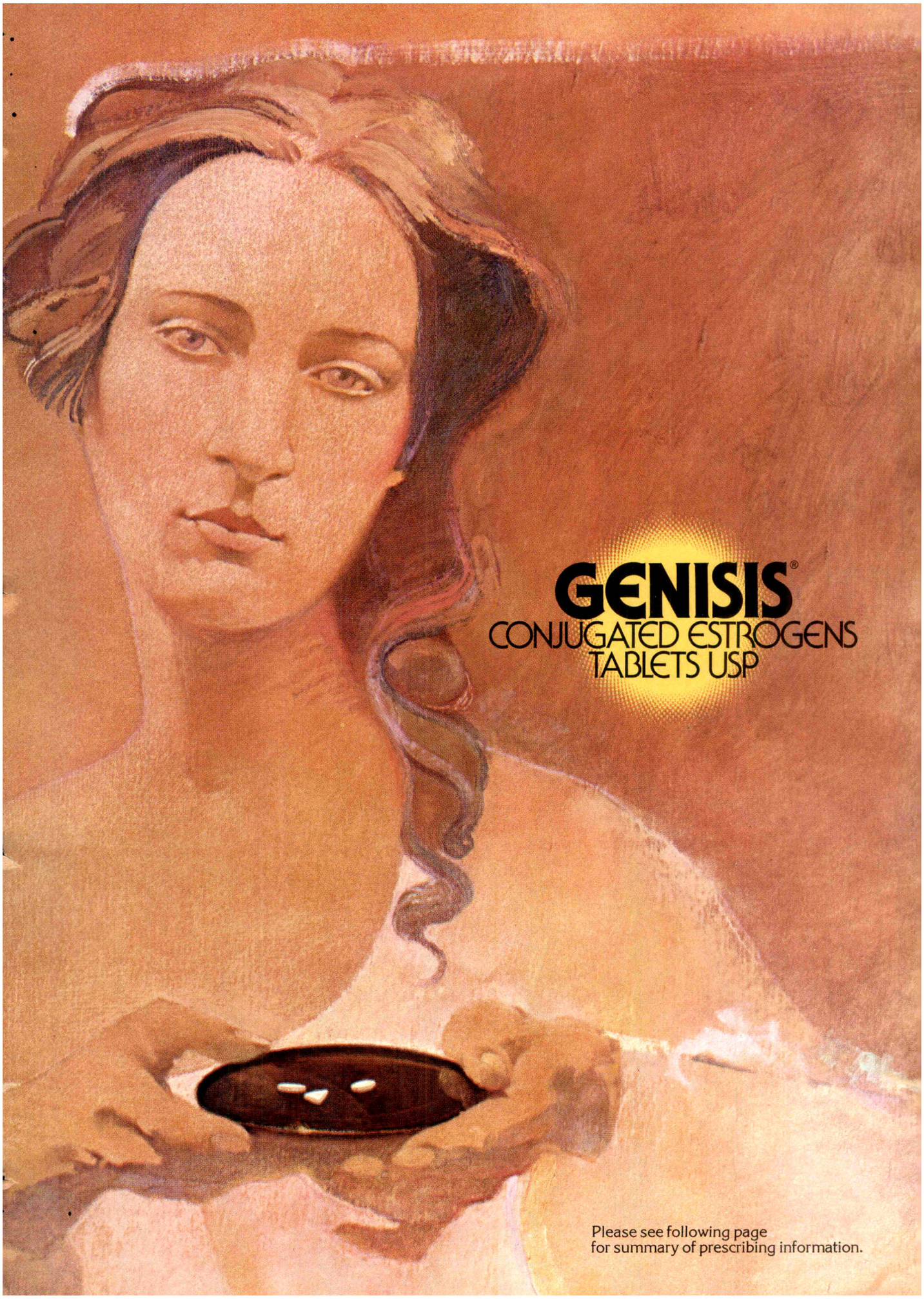
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Genisis is supplied in snow-white pure uncoated tablets in the usual potencies of: 0.625 mg/1.25 mg/2.50 mg.


GENISIS®
CONJUGATED ESTROGENS
TABLETS USP
Please see following page
for summary of prescribing information.

GENISIS®
CONJUGATED ESTROGENS TABLETS USP

BRIEF SUMMARY
Please consult full package circular before prescribing

Indications
Genisis is indicated for replacement therapy of estrogen deficiency associated with menopausal syndrome, female hypogonadism (hypogenitalism), amenorrhea, female castration or primary ovarian failure.

Important Notes on the Use of Estrogens
In view of the positive relationship between thromboembolic disease and the use of oral contraceptives containing estrogens and progestins, it would seem prudent and in keeping with basic therapeutic principles to utilize, whenever feasible, the smallest effective dose of estrogens in treating patients.

The possible carcinogenicity of estrogens cannot be confirmed or refuted at this time. Close clinical surveillance of all women taking estrogens must be continued.

Contraindications
Patients with, or with history, of:
markedly impaired liver function;
known or suspected carcinoma of breast (except progressing disease not amenable to surgery or irradiation in women at least 5 years postmenopausal, or in men in whom castration is not possible);
known or suspected estrogen-dependent neoplasia, such as carcinoma of the endometrium;
undiagnosed genital bleeding;
thrombophlebitis, thromboembolic disorders, cerebral apoplexy.

Warnings
Discontinue medication at the earliest sign of thrombotic disorders (thrombophlebitis, cerebrovascular disorders, pulmonary embolism, retinal thrombosis), or in the presence of papilledema, retinal vascular lesions or such untoward effects as progression of cancer or hypercalcemia. Discontinue pending examination if there is sudden onset of proptosis, diplopia or migraine.

A statistically significant association has been reported between maternal ingestion of diethylstilbestrol during pregnancy and the occurrence of vaginal carcinoma years later in the offspring. Whether such an association is applicable to all estrogens is not known at this time. In any event, estrogens are not indicated for use during pregnancy.

Estrogens excreted in mother's milk may affect the nursing infant.

Precautions
Certain patients may be unusually responsive and may respond with undesirable manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding, mastodynia, edema, etc. Because of possible salt and water retention, estrogens should be used with caution in patients with epilepsy, migraine, asthma, cardiac or renal disease. Estrogens influence the metabolism of calcium and phosphorus. They should be used with caution in patients with certain metabolic bone diseases that are associated with hypercalcemia or in patients with renal insufficiency. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Diabetic patients should be followed closely because of possible decrease in glucose tolerance.

In the event of unexplained or excessive vaginal bleeding during estrogen therapy, nonfunctional causes should be borne in mind. The drug should be discontinued and a thorough investigation made as to the cause, being certain to rule out the possibility of malignancy. Pre-existing uterine fibromyomata may increase in size when estrogens are used. Therefore, patients should be examined at regular intervals throughout therapy. Women with a strong family history of cancer, recurrent chronic mastitis, or abnormal mammograms should be administered estrogens with caution. A pre-treatment physical examination should include special reference to breast and pelvic organs as well as a Papanicolaou smear since estrogens have been known to produce tumors, some of them malignant, in five species of animals.

Continuous use of estrogens will result in prolonged stimulation of the endometrium and breast. In order to avoid this, oral estrogens should be administered cyclically in the menopausal or hypogonadal patient.

The pathologist should be advised of estrogen therapy when relevant specimens are submitted. Certain endocrine and liver function tests may be affected by treatment with estrogens. If such tests are abnormal in a patient taking these drugs it is recommended that they be repeated after the drug has been withdrawn for two months. Any possible influence of prolonged estrogen therapy on pituitary, ovarian, adrenal, hepatic or uterine function awaits further study.

When large doses of estrogens are used, urinary stress incontinence may occur in nonpregnant females.

Adverse Reactions
A statistically significant association has been demonstrated between use of estrogen-progestin oral contraceptives and the following serious reactions: thrombophlebitis, cerebral thrombosis, pulmonary embolism.

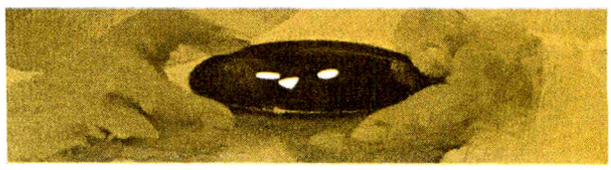
Although available evidence is suggestive of an association, such a relationship has been neither confirmed nor refuted for the following serious reactions: coronary thrombosis, neuro-ocular lesions (e.g. retinal thrombosis and optic neuritis).

The following adverse reactions are known to occur in patients receiving estrogens: nausea, vomiting, anorexia, gastrointestinal symptoms (such as abdominal cramps or bloating), edema, breakthrough bleeding, spotting or withdrawal bleeding, breast tenderness and enlargement, change in body weight (increase or decrease), headache, increased cervical mucus, allergic rash, loss of libido and gynecomastia in the male, reactivation of endometriosis, aggravation of migraine headaches, hepatic cutaneous porphyria becoming manifest, cholestatic jaundice, rise in blood pressure in susceptible individuals, mental depression, cystitis-like syndrome, loss of scalp hair, erythema nodosum, hemorrhagic eruption, premenstrual-like syndrome, changes in libido, changes in appetite, nervousness, dizziness, fatigue, backache, erythema multiforme, itching, possible diminution in lactation when given immediately post-partum, irritability, malaise.

Dosage and Administration
Dosage requirements will vary with the severity of menopausal symptoms. Initially, doses ranging from 1.25 mg to 3.75 mg daily may be required to bring symptoms under control. When this has been accomplished dosage should then be gradually decreased until the minimal effective maintenance dose is established. This will usually be 0.625 mg or 1.25 mg daily or possibly less.

Caution
Federal law prohibits dispensing without a prescription.

How Supplied—0.625 mg tablets—bottles of 100 and 500
1.25 mg tablets—bottles of 100 and 500
2.5 mg tablets—bottles of 100



References
1. Savard, K.: The estrogens of the pregnant mare. Endocrinology 68:411, 1961.
2. Howard, R.P. and Keaty, E.C.: Evaluation of equilin 3-monosulfate and other estrogens. Arch. Int. Med. 128:229, 1971.
3. Goodman, L.S. and Gilman, A.: The pharmacological basis of therapeutics. New York, MacMillan Pub. Co. Inc., 1975, p. 1451.

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A SYMPOSIUM ON FETAL MONITORING

The Symposium will be held at the Château Frontenac in Quebec City, Canada, from Wednesday, April 21st to Saturday, April 24th 1976. The three days will be devoted to the study of recent advances in monitoring the fetal heart and uterine contraction. Internationally known experts in these fields will be giving presentations. Lectures and workshops have been organised for physicians and nurses interested in the subjects.

Official languages will be English and French with simultaneous translation available.

Registration will be closed April 1st 1976. The regular fee will be: physicians, one hundred and fifty canadian dollars ($150.00); nurses, fifty dollars ($50.00), including three breakfasts and two lunches at the Château Frontenac. For registration after April 1st, the fee will be: physicians, two hundred dollars ($200.00); nurses, seventy-five dollars ($75.00). Cheques should be made payable to: Symposium de monitorage foetal, Département d'Obstétrique-Gynécologie, Faculté de Médecine, Cité Universitaire, Québec, Canada, G1K 7P4.

For further information write to: Adrien Bastide M.D., Hôpital Saint-Francois d'Assise, 10 de l'Espinay, Québec, Canada, G1L 2H1 or call (418) 688-8710.



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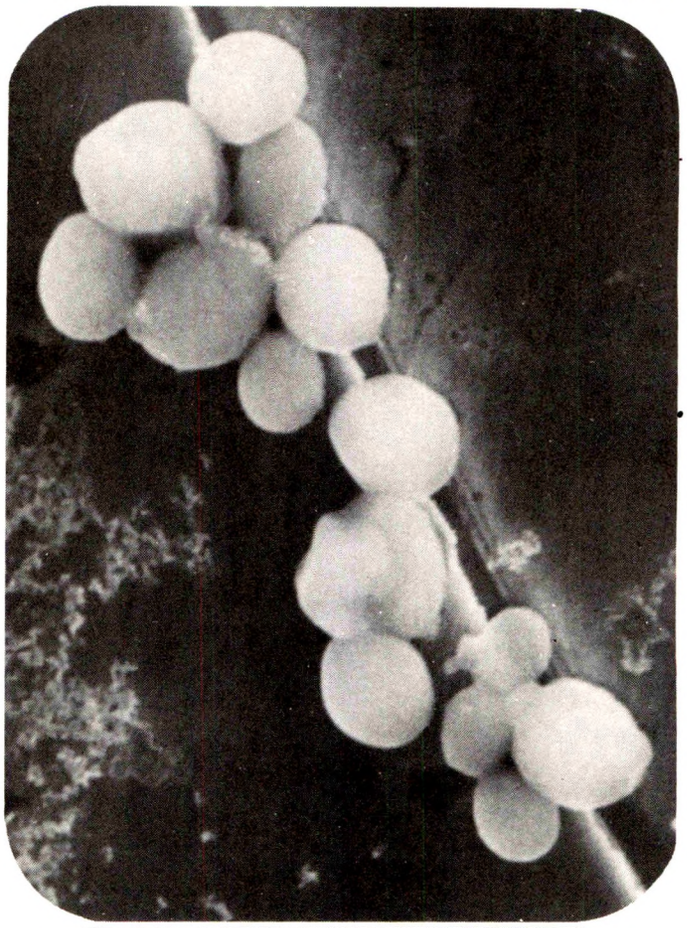
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SEM photomicrograph (5,300×) *Candida albicans, in vitro* control

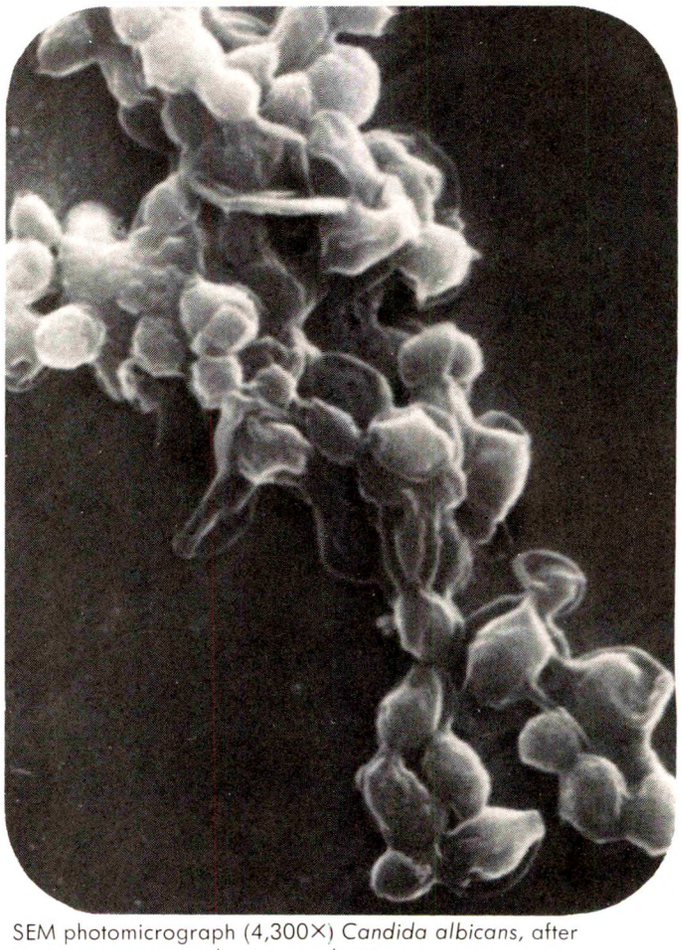


the first new agent for vaginal moniliasis in over 10 years...

effective when used once daily, h.s., for 14 days†

NOTE: Clinical studies have shown the efficacy of MONISTAT Cream in humans. The above Scanning Electron Microscope photographs represent *in vitro* research. No clinical significance should be drawn from this study concerning the effectiveness of miconazole nitrate, as it is not possible to extrapolate *in vitro* data to humans.

†Recommended dosage is once daily, h.s. for 14 days.



SEM photomicrograph (4,300×) *Candida albicans*, after *in vitro* treatment with miconazole nitrate

significantly high cures after one course of therapy

In a controlled study,[1] a total of 418 patients met the *rigid* criteria for inclusion. A cure was defined as complete relief of vulvar pruritus, burning and irritation plus negative KOH smear and negative NICKERSON'S* Medium Culture 30 days or more after completion of therapy.

Results show effectiveness in a wide range of patients

In clinical studies, new MONISTAT Cream produced outstanding cures in:

1. Pregnant†† and non-pregnant patients.
2. Patients on antibiotics or hormonal therapy and
3. Patients previously treated, one or more times with other antifungal drugs.

Monistat Cream is a soothing, non-staining, water-miscible cream

In addition to high cure rate, patients readily accept MONISTAT because...

Symptomatic relief for majority of patients by third application

that permits rapid contact with infected tissues and may be used on the vulva.

Convenient, once-a-day bedtime dosage

does not interfere with daytime activities.

Full 14-day course of therapy in one tube.

For the majority of patients this is sufficient to eliminate the infection.

[1]Clinical Compendium December 1973, Ortho Pharmaceutical Corporation, Raritan, New Jersey.

††MONISTAT Cream may be used in the second and third trimester. However, in the first trimester, it should be used only when the physician considers it essential to the patient's welfare. See precautions in prescribing information.

MONISTAT Cream

TRADEMARK

(MICONAZOLE NITRATE 2%)

Prescribing Information

Description MONISTAT (miconazole nitrate 2%) is a water miscible, white cream containing as the active ingredient, 2% miconazole nitrate, 1-[2,4-Dichloro-β-(2,4-dichlorobenzyloxy) phenethyl] imidazole nitrate.

Actions MONISTAT Cream exhibits fungicidal activity *in vitro* against species of the genus *Candida*. The pharmacologic mode of action is unknown.

Indications MONISTAT Cream is indicated for the local treatment of vulvovaginal candidiasis (moniliasis).

As MONISTAT Cream is effective only for candidal vulvovaginitis, the diagnosis should be confirmed by KOH smears and/or cultures. Other pathogens commonly associated with vulvovaginitis (Trichomonas and *Haemophilus vaginalis*) should be ruled out by appropriate laboratory methods.

MONISTAT is effective in both pregnant and non-pregnant women, as well as in women taking oral contraceptives. (See Precautions.)

Contraindication Patients known to be hypersensitive to this drug.

Precautions *General:* Discontinue drug if sensitization or irritation is reported during use. *Laboratory Tests:* If there is a lack of response to MONISTAT, appropriate microbiological studies should be repeated to confirm the diagnosis and rule out other pathogens.

Pregnancy: MONISTAT Cream was used in 209 pregnant patients. Follow-up reports now available in 117 of these patients reveal no adverse effects or complications attributable to MONISTAT therapy in infants born to these women.

Since MONISTAT Cream is absorbed in small amounts from the human vagina, it should be used in the first trimester of pregnancy only when the physician considers it essential to the welfare of the patient.

Adverse Reactions Thirty-nine (7.4%) of the 528 patients treated with MONISTAT reported complaints during therapy that were possibly drug-related. Most complaints were reported during the first week of therapy. Vulvovaginal burning, itching or irritation occurred in 6.6%, while other complaints such as vaginal burning, pelvic cramps, hives, skin rash and headache occurred rarely (each less than 0.2% patient incidence). The therapy-related drop-out rate was 0.9%.

Dosage and Administration One applicatorful is administered intravaginally once daily at bedtime for 14 days. Course of therapy may be repeated after other pathogens have been ruled out by appropriate smears and cultures.

Supplied MONISTAT Cream is available in 3 oz. (85 g) tubes with Ortho* Measured Dose Applicator.

MONISTAT (miconazole nitrate 2%) is a development of Ortho Research Foundation and Janssen Pharmaceutica, Belgium.

*TRADEMARK

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OJ 560-4

Ortho Pharmaceutical Corporation, Raritan, New Jersey 08869

Abnormal uterine bleeding: progesterone replacement... to oppose unopposed estrogen

Estrogen replacement therapy... a frequent cause of abnormal uterine bleeding

Administration of estrogen for the relief of menopausal symptoms is a frequent cause of irregular bleeding or spotting during the menopause, and the most common cause of uterine bleeding after the menopause. Bleeding may occur when long-term administration of estrogen causes overstimulation of the endometrium.

It cannot be assumed, however, that uterine bleeding in a woman on estrogen replacement therapy is due solely to the exogenous estrogen. The existence of a pathologic condition—benign or malignant—should always be considered, thoroughly explored, and ruled out. It is generally agreed that any woman taking estrogen who complains of abnormal uterine bleeding should undergo a careful physical examination including a Pap test, followed by curettage of the uterine cavity.

A close look at the influence of estrogen and progesterone on the endometrium

In the normal proliferative endometrium (left), the epithelium contains rather flat, nonciliated cells with small microvilli, and scattered, uniform ciliated cells. Regrowth of the surface epithelial cells proceeds vigorously throughout the proliferative phase of the cycle. (X2250)

Under the continued and unopposed influence of estrogen (center), there is a striking increase in the number of ciliated cells and in the abundance and size of the microvillous processes on the nonciliated cells. (X1600)

During the normal secretory phase (right), the presence of progesterone causes the secretory cells to become more complex, and the microvilli to increase in number. Scattered droplets are visible on the surface of some of the nonciliated cells and are attached to the tips of some microvilli. (X2500)



Scanning electron micrographs courtesy of Ralph M. Richart, M.D.

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rovera replacement
) oppose unopposed estrogen

'hen the unopposed action of exogenous estro-
n is responsible for abnormal uterine
eeding in the menopausal or postmenopausal
tient, administration of Provera®
iedroxyprogesterone acetate), 5 mg - 10 mg
ily for 5-10 days, may be all that
ieeded to control or regulate bleeding.
overa, an orally active derivative of
ogesterone, transforms the estrogen-primed
dometrium into a secretory endometrium.
eeding and rapid shedding of the
dometrium occur following cessation of
ogestin therapy.

recautions and
ontraindications

overa appears to be devoid of significant
trogenic activity; however, andro-
nic effects have been noted with the drug.
uring progesterone therapy, thrombophlebitis
d pulmonary embolism have been reported.
r this reason, such therapy is contra-
dicated in women with a history of these
cidents. Progesterone therapy is also
contraindicated in the presence of liver dysfunction or disease, known or suspected malignancy of breast or genital organs, undiagnosed vaginal bleeding, and missed abortion. Because a decrease in glucose tolerance may occur during Provera therapy, diabetic patients should be followed closely. Patients with a history of psychic depression should be carefully observed and if serious depression occurs, Provera should be discontinued.

To control abnormal uterine bleeding in the absence of organic pathology

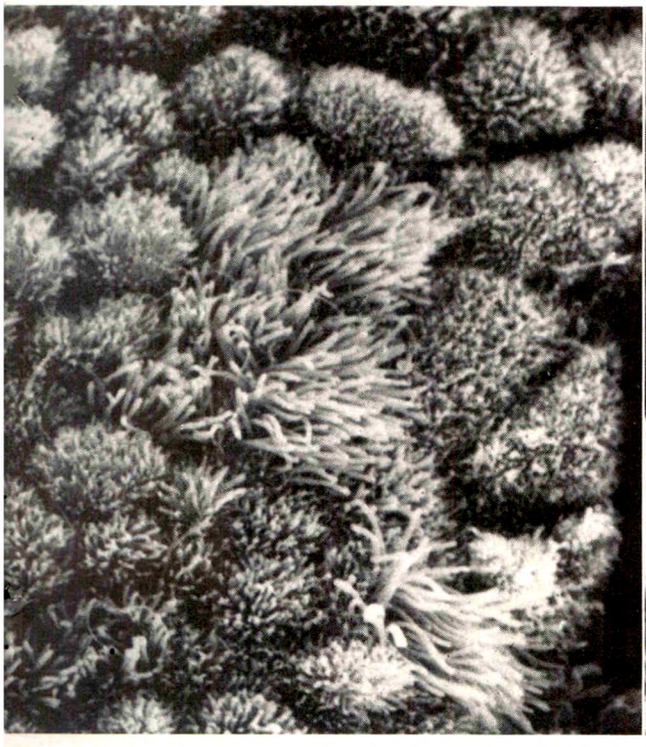
Provera® 10 mg Medroxyprogesterone Acetate Tablets, U.S.P., Upjohn

Upjohn

The Upjohn Company, Kalamazoo, Michigan 49001

Please turn the page for additional prescribing information.

September, 1974 J-4113-4





To control abnormal uterine bleeding in the absence of organic pathology

Provera® 10 mg Medroxyprogesterone Acetate Tablets, U.S.P., Upjohn

Description and actions: Medroxyprogesterone acetate is a derivative of progesterone. Administered orally or parenterally to women with adequate endogenous estrogen it transforms proliferative endometrium into secretory endometrium.

Indications: Provera Tablets are indicated in secondary amenorrhea and abnormal uterine bleeding due to hormonal imbalance in the absence of organic pathology such as fibroids or uterine cancer.

Contraindications: Thrombophlebitis, thromboembolic disorders, cerebral apoplexy or patients with a past history of these conditions. Liver dysfunction or disease. Known or suspected malignancy of breast or genital organs. Undiagnosed vaginal bleeding. Missed abortion. Known sensitivity to medroxyprogesterone acetate.

Warnings: 1. Immediately discontinue administration should any of the following thrombotic disorders occur or be suspected: thrombophlebitis, cerebrovascular disorders, pulmonary embolism, retinal thrombosis. **2.** Beagle dogs treated with medroxyprogesterone acetate developed mammary nodules, some of which were malignant. Although nodules occasionally appeared in control animals, they were intermittent in nature, whereas the nodules in the drug treated animals were larger, more numerous, persistent, and there were some breast malignancies with metastases. Their significance with respect to humans has not been established. **3.** Discontinue medication pending examination if there is sudden partial or complete loss of vision, or if there is a sudden onset of proptosis, diplopia or migraine. If examination reveals papilledema or retinal vascular lesions, medication should be withdrawn. **4.** Detectable amounts of progestin have been identified in the milk of mothers receiving the drug. The effect of this on the nursing infant has not been determined. **5.** Usage in pregnancy is not recommended because of data indicating a possible association between administration of progestins early in pregnancy and congenital heart defects in the offspring. **6.** Three major studies in Great Britain and one in this country have shown a statistically significant association between thrombophlebitis, pulmonary embolism, cerebral thrombosis and embolism and the use of oral contraceptives. It has been estimated that users are several times as likely to undergo thromboembolic disease without evident cause as nonusers. The American study indicated that the risk did not persist after discontinuation and it was not enhanced by long continued administration.

Precautions: A pretreatment physical exam should include special reference to breast and pelvic organs and a Papanicolaou smear. This drug may cause some degree of fluid retention; therefore, observe carefully patients with conditions influenced by fluid retention such as epilepsy, migraine, asthma, and cardiac or renal dysfunction. In breakthrough bleeding, and other irregular bleeding per vaginum bear in mind nonfunctional causes. Perform adequate diagnostic measures in cases of undiagnosed vaginal bleeding. Advise pathologist of therapy when submitting relevant specimens. Carefully observe patients with history of psychic depression and discontinue drug if serious degree of depression recurs. Any possible influence of prolonged therapy on pituitary, ovarian, adrenal, hepatic, or uterine function awaits further study. Decreased glucose tolerance has been observed in a small percentage of patients on estrogen progestin combinations, therefore, carefully observe diabetic patients receiving progestin therapy. Age constitutes no absolute limiting factor, although onset of climacteric may be masked. Because of the occasional occurrence of thrombotic disorders (thrombophlebitis, pulmonary embolism, retinal thrombosis, and cerebrovascular disorders) in patients taking estrogen-progestin combinations and since the mechanism is obscure, the physician should be alert to the earliest manifestation of these disorders.

Adverse reactions: The following have been reported: breast tenderness or galactorrhea in a few instances; an inferential association between the administration of progestins early in pregnancy and the occurrence of congenital malformations (see also Warnings); occasional sensitivity reactions including pruritus, urticaria, edema and generalized rash; acne, alopecia and hirsutism in a few patients; thromboembolic phenomena including thrombophlebitis and pulmonary embolism.

The following adverse reactions have been observed in women taking progestins including medroxyprogesterone acetate: breakthrough bleeding; spotting, change in menstrual flow; amenorrhea; edema, increase or decrease in weight; changes in cervical erosion and secretions; cholestatic jaundice, rash (allergic) with and without pruritus, and mental depression.

A statistically significant association has been demonstrated between use of estrogen-progestin combination drugs and the serious adverse reactions of thrombophlebitis, pulmonary embolism and cerebral thrombosis and embolism. Therefore, patients on progestin therapy should be carefully observed.

Although available evidence is suggestive, a relationship has been neither confirmed nor refuted for the association of the serious adverse reaction of neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been observed *in* patients receiving estrogen-progestin combination drugs: rise in blood pressure in susceptible individuals; premenstrual-like syndrome, changes in libido; changes in appetite, cystitis-like syndrome, headache; nervousness; dizziness; fatigue; backache, hirsutism; loss of scalp hair; erythema multiforme; erythema nodosum; hemorrhagic eruption; and itching. Therefore, observe patients on progestin therapy carefully.

The following laboratory results may be altered by the use of estrogen-progestin combination drugs: increased sulfobromophthalein and other hepatic function tests; coagulation tests (increase in prothrombin factors VII, VIII, IX and X), metyrapone test; pregnanediol determination; thyroid function tests (increase in PBI, and butanol extractable protein bound iodine and decrease in T^3 uptake values).

How supplied: *2.5 mg* in bottles of 25. *10 mg* in bottles of 25 and 100.

Caution: Federal law prohibits dispensing without prescription

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OBSTETRICS

Effects of progesterone, prostaglandin $F_{2\alpha}$ and its analogue ICI 81008 on the excitability and threshold of the uterus

A. I. CSAPO*

St. Louis, Missouri

Forty-eight uterine strips were excised from 24 New Zealand white rabbits and studied in vitro. Sixteen strips were examined when eight animals were 25 days pregnant, 24 strips when 12 animals were about 12 hours postpartum, and eight strips about 12 hours after four animals received a single dose of 5 mg. progesterone (P) at spontaneous delivery. When after repeated exposures to electric fields (60 cycles per second, alternating current, of optimum strength, four seconds' duration, at intervals of 30 seconds) the ~ 0.5 Gm. strips developed ~ 40-50g wall tension (WT) in steady state, the field strength was reduced in graded steps from 12 v per 5 cm. to 10, 8, 6, 4 and 3 v per 5 cm. and WT recorded. By measuring the effect on WT of "decreasing electric field stimulation" (DEFS), myometrial excitability and threshold were characterized and quantitated. The technique is based on the relationship between the fraction of myometrial cells activated in the total cell population and WT. As the stimulus is decreased in graded steps from optimal to increasingly suboptimal, DEFS activates fewer and fewer cells reflected by the decrease in WT. Threshold is defined by the strength of the minimum electric field which provokes WT and subthreshold by the field strength which fails to excite. Thus fractional WT averages the excitability of cells in the tissue and thus DEFS characterizes this important parameter of uterine function and quantitates threshold under a variety of regulatory conditions. The comparative study of excised uteri in three different endocrine states showed that the regulatory conditions of normal pregnancy (P levels 9 ng. per milliliter) suppressed excitability and increased threshold (to 6 v per 5 cm.). Post partum (P levels 1 ng. per milliliter) excitability increased and threshold decreased (to 4 v per 5 cm.), unless P treatment prevented this characteristic change by suppressing excitability and increasing threshold (to 6 v per 5 cm.). Both regulatory agents, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and its analogue ICI 81008, increased excitability and decreased threshold significantly in picogram per milliliter concentrations. However, this effect was observed only when the P levels were low. These compounds had little effect, even in nanogram per milliliter concentrations, when the P levels were high. The studies also revealed a close relationship between threshold and spontaneous uterine activity. Low threshold promoted while high threshold suppressed spontaneous activity. In similar concentrations the oxytocic actions of $PGF_{2\alpha}$ and ICI 81008 were similar but the effect of ICI 81008 was prolonged through strong binding at myometrial sites.

From the Department of Obstetrics and Gynecology, Washington University School of Medicine.

Supported by the Agency for International Development, Department of State, Contract No. AID/csd 3160.

Received for publication November 25, 1974.

Accepted February 20, 1975.

Reprint requests: Dr. A. I. Csapo, Department of Obstetrics and Gynecology, 4911 Barnes Hospital Plaza, St. Louis, Missouri 63110.

**Research Career Awardee of the CPR, National Institute of Child Health and Human Development, No. 5-K6-HD-20, 169.*

Intrauterine pressure (IUP) tracings, obtained during the pioneering trials with prostaglandins (PG's), showed explicitly[1] that these compounds trigger in the uterus normal oxytocic responses (for example, advanced cyclic IUP) only when gestation approaches term, while during pregnancy they provoke unphysiologic contractures. On these contracture responses advanced cyclic IUP (approximating the maximum working capacity of the uterus, described by LaPlace: IUP = 2WT/R*) was superimposed only after several hours' delay. These findings had been complemented by the additional demonstrations[2] that: (1) before a normal oxytocic response can be triggered by PG's (or other stimulants), the pregnant uterus must be "converted" (endogenously or exogenously) from a refractory to a reactive organ; (2) this characteristic and time-dependent regulatory change ("conversion") can be provoked by progesterone withdrawal (Pw); (3) conversion does occur regardless of how Pw is provoked, for example, by the endogenous mechanism,[3-5] by luteectomy,[6] by hypertonic saline,[7, 8] or by PG's.[9-16]

Despite this multiple evidence demonstrating that the abortifacient efficacy of PG's is dependent more on their potency to provoke conversion than oxytocic response, in some centers PG's were given in prolonged infusions or repeated small doses in an attempt to achieve sustained uterine stimulation. PG therapy by these delivery systems yielded unsatisfactory clinical results (*see* Csapo[2]). Other centers adopted the therapeutic principle: "PG impact" (PGI, a massive intrauterine PG dose) which did provoke uterine conversion, presumably by suppressing uterine microcirculation and fetoplacental endocrine function, resulting in Pw.[20-22] In provoking Pw, the PGI improved abortifacient therapy,[9-16] since the regulatory imbalance induced promoted the evolution of spontaneous uterine activity and clinical progress in cervical dilatation and abortion.

However, several questions remained to be answered. It had to be explained how Pw (a decrease in a suppressor) can increase the "sensitivity" of the uterus to stimulants, indeed bring about spontaneous activity. The studies[2, 17-19] revealed that: (1) the intrinsic myometrial stimulant is PG, present in the uterus at all times even under in vitro conditions; (2) when PG synthesis is blocked PG can, while oxytocin cannot, stimulate the uterus; (3) even PG's fail in provoking advanced cyclic IUP during pregnancy when normal P levels sustain high threshold; (4) PG-synthesis in the uterus is stretch dependent. Thus the activation of the pregnant uterus by Pw had been explained by a "shift" in regulatory balance[20] induced through an endogenously or therapeutically provoked dominance of PG over P in the control of threshold.

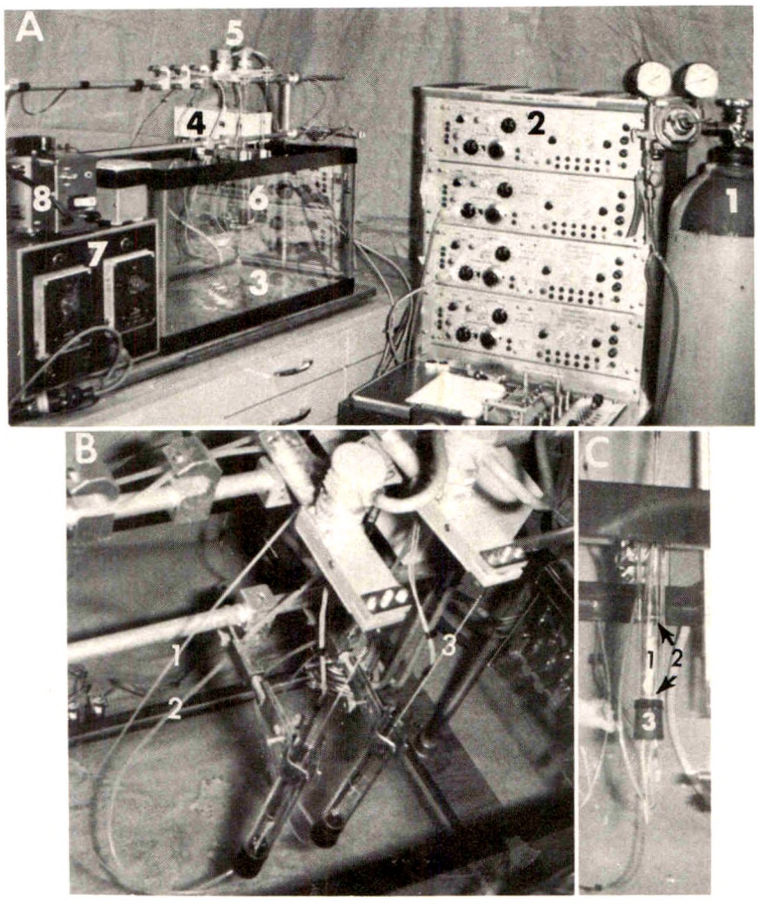
This re-examination of the regulatory mechanism[2-18] exposed the significance of threshold control in reproductive medicine. Once exposed, techniques had to be developed for studying it—specifically the effects of PG's and P on threshold and the relationship between threshold and spontaneous uterine activity and reactivity. Furthermore, it had to be determined whether the increased efficacy of PG analogues (exceeding those of the natural compounds) is due to their increased oxytocic potency or their binding at target sites, complemented with reduced metabolism. The present study is an attempt in answering some of these and related questions.

Material and methods

Technical considerations. Excised strips from pregnant uteri contain thousands of spindle-shaped myometrial cells, each of them approximately 100μ long and about 5 to 10μ wide. Extensive studies with intracellular microelectrodes revealed[23, 24, 27] considerable variations in the membrane and action potentials of these cells (regardless of their regulatory condition), indicating variations in the threshold of individual cells and in the pacemaker and conduction characteristics of different uterine regions. To reduce uncertainties, a method had to be developed which could characterize excitability and quantitate threshold by "averaging" the properties of all cells of the excised myometrium and relate the excitability and threshold to the spontaneous activity of the uterus in a variety of regulatory conditions. The technique of "decreasing electric field stimulation" (DEFS), used in earlier studies,[2, 17] promised a reliable method. The present experiments explored the potential of this technique for characterizing the regulatory and functional conditions of the uterus by defining threshold through the minimum electric field which provoked a tension response and excitability through a series of tension responses induced by a standard set of suboptimal electric stimuli.

The preparation of uterine strips and their transfer to in vitro conditions. The rabbits were killed by a blow on the head and opening of the carotid artery. One uterine horn was quickly transferred to oxygenated (95 per cent O_2 + five per cent CO_2) mammalian Krebs' solution. From this horn a 3 cm. long strip was removed with intact longitudinal fiber bundles and slit into twin portions, each approximately 30 mm. long,

*Where IUP = intrauterine pressure, WT = wall tension, and R = uterine radius.[23]



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Fig. 1. The experimental arrangement for the study of maximum tension, the effect on tension of decreasing electric field stimulation (DEFS), and the action of drugs on excitability and threshold. Photographs of the experimental arrangement: *A—1*, O_2 Tank; *2*, Grass polygraph; *3*, constant-temperature water bath; *4*, temperature control; *5*, force-displacement transducer; *6*, muscle chamber; *7*, Tandem automatic stimulator; *8*, rheostat. *B—1*, O_2 inlet; *2*, Krebs' inlet; *3*, silver chain, connecting muscle with transducer; *C—1, the uterine strip; 2*, pair of platinum ring stimulating electrodes; *3*, rubber stopper.

about 4 mm wide, and of natural thickness. Both strips were suspended isometrically in oxygenated Krebs' solution at 37° C. and pH 7.4 and stretched until they sustained ~ 2g resting tension ("optimum length"[23]). Since these strips were removed from different portions of the uterus in between the ovarian and cervical regions they represented the properties of the uterus as a whole. However, during pregnancy the placental implantation sites were avoided. The uterine wall thickness (W) of six 25 days' pregnant and six postpartum strips (measured at three different points in histologic cross-sections) were: 1.6 ± 0.1 and 1.7 ± 0.2 mm., respectively (of which the myometrial thickness was 0.9 ± 0.1 and 1.1 ± 0.1 mm.), $p > 0.05$).

The experimental arrangement, electric stimulation, maximum tension and threshold. Fig. 1 describes the experimental arrangement used. It consisted of: a pyrex tube closed at its lower end with a rubber stopper; two polyethylene tubes for the delivery of Krebs' solution and O_2 through the rubber stopper; a pair of platinum ring electrodes (5 cm.apart) for periodic stimulation of the uterus inside the Krebs' solution; two platinum hooks and a silver chain for isometric mounting of the uterus; an automatic (Tandem) timer for the delivery of four seconds' long electric stimuli in every 30 seconds; a rheostat for altering the strength of the electric field; and a Grass force-displacement transducer (Model FT03C) and polygraph (Model 5C) for the recording of tension. When twin strips were studied simultaneously, two muscle chambers were used.

Fig. 2 illustrates that at the beginning of the study the postpartum uterus developed stepwise increasing tension when stimulated repeatedly with an "optimum"

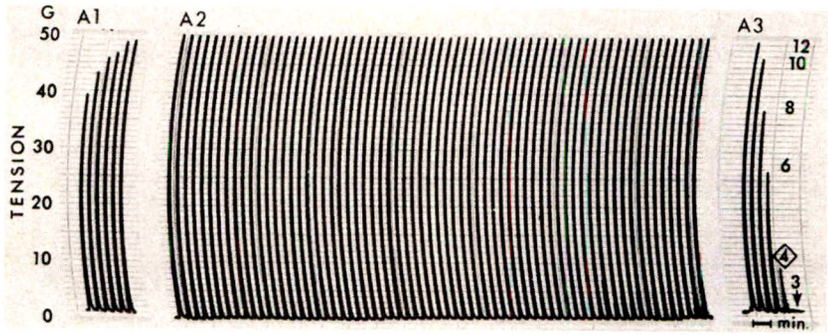


Fig. 2. Study of the effects of DEFS. Uterus: approximately 12 hours' postpartum. *A1*, The build up of uterine wall tension (WT) with 12 v. per 5 cm. field. *A2*, Maximum WT in a steady state. *A3*, The effect of stepwise "decreasing electric field stimulation" (DEFS) and the assessment of "threshold" (in this instance 4 v. per 5 cm.). For further description see text.

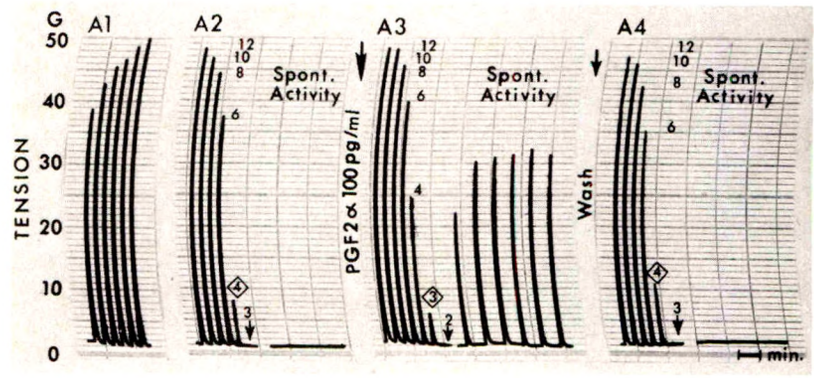


Fig. 3. The effect of $PGF_{2\alpha}$ on threshold and spontaneous activity. Uterus: approximately 12 hours' postpartum. *A1*, The build up of WT with 12 v. per 5 cm. field. *A2*, The effect of DEFS, the assessment of threshold (4 v. per 5 cm.), and spontaneous activity (none). *A3*, The lowering of threshold with 100 pg. per milliliter $PGF_{2\alpha}$ (to 3 v. per 5 cm.) and the initiation of spontaneous activity. *A4*, Removal of $PGF_{2\alpha}$ by washing, increase of threshold (to 4 v. per 5 cm.), and suspension of spontaneous activity.

field of 12 v. per 5 cm. (60 cycles per second, alternate current, during four seconds in every 30 seconds). Maximum tension was reached within three to five minutes (*A1*). Subsequently tension remained steady and maximal at ~ 50g (*A2*), although the weight of the strip was only about 0.5 Gm.

Having determined maximum tension and recorded it in a steady state, the effect of stepwise "decreasing electric field stimulation" (DEFS) was examined with the use of:12, 10, 8, 6, 4 and 3 v. per 5 cm. fields (*A3*). The decreasing field activated fewer and fewer cells of the total cell population resulting in stepwise decrease in WT. The subthreshold stimulus (3 v. per 5 cm.) failed to provoke WT altogether. The minimum field (4 v. per 5 cm.) which still triggered WT was called the threshold stimulus (*A3*), and thus by definition the threshold of this uterus was 4 v. per 5 cm.

The effect of $PGF_{2\alpha}$ on threshold and spontaneous activity of the uterus. Fig. 3 illustrates that after maximum tension had been built up with a series of 12 v. per 5 cm. stimuli (*A1*), DEFS was applied to characterize excitability and threshold of this approximately 12 hours postpartum uterus. Within the range of 12 to 6 v. per 5 cm. WT decreased only slightly, indicating high excitability. Threshold was low at 4 v. per 5 cm. and the subthreshold stimulus (which failed to excite) 3 v. per 5 cm. However, when stimulation was discontinued, no spontaneous activity appeared during ~ 5 minutes indicating that under the present experimental conditions the intrinsic stimulatory mechanism could not break through threshold (*A2*). Only when the same strip was exposed to 100 pg. per milliliter of $PGF_{2\alpha}$, which decreased its threshold to 3 v. per 5 cm., did spontaneous activity appear promptly on the discontinuation of electric stimulation (*A3*). Apparently $PGF_{2\alpha}$ lowers threshold, increases excitability, and in so doing promotes spontaneous uterine activity. Washing the strip with Krebs' solution removed $PGF_{2\alpha}$,

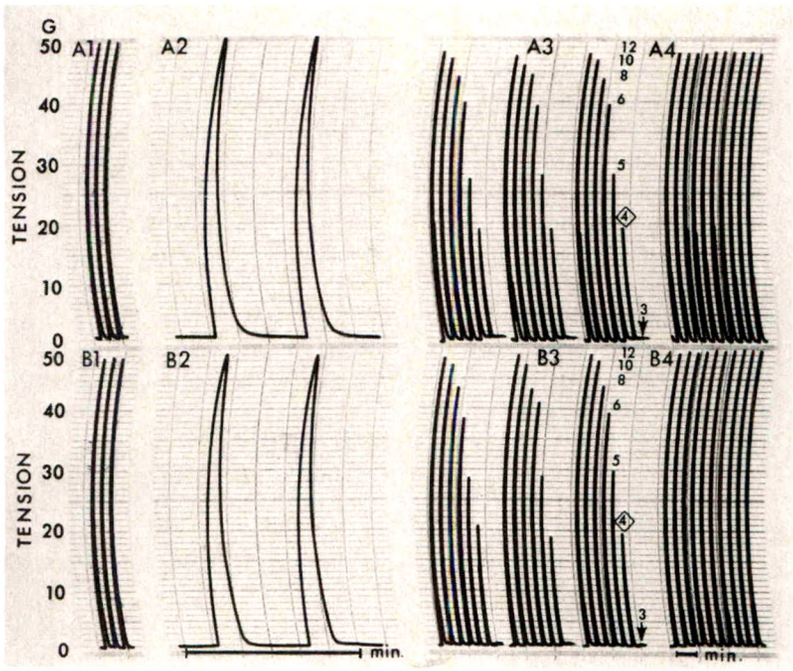


Fig. 4. The stability of excitability and threshold in twin strips. Uterus: approximately 12 hours postpartum. A simultaneous study of twin strips under identical experimental conditions. *A1* and *B1*, Maximum WT induced by 12 v. per 5 cm. field. *A2* and *B2*, The shape of the contraction cycles. *A3* and *B3*, The stability of excitability and threshold during three subsequent studies. *A4* and *B4*, Maximum WT in steady state at the end of the experiment.

increased threshold to the pretreatment value (4 v. per 5 cm.), and suspended spontaneous activity (*A4*).

The reliability of the method. Using twin (rather than single) strips of the same uterus the reliability of the method had been determined in preparation to comparative studies of excitability and threshold under a variety of regulatory conditions. Fig. 4 illustrates that when geometrically similar* twin strips (of the same uterus) were stimulated at optimum field strength (12 v. per 5 cm.) WT was maximal and steady (*A1* and *B1*) and the shape of the contraction-relaxation cycles comparable (*A2* and *B2*). Furthermore, the response of the strips to a series of DEFS was similar and threshold constant (4 v. per 5 cm.) during three subsequent studies (A3 and B3). In addition, 12 v. per 5 cm. triggered maximum WT in a steady state at the end of the experiment (*A4* and *B4*), further illustrating the stability of the uterine strips and the reliability of the technique.

Apparently a method (DEFS) had been developed which, in uterine strips of different regulatory background, can: (1) characterize excitability by the rate of decline in WT on DEFS; (2) quantitate threshold by the minimum field which provokes WT; (3) determine the regulatory action of various agents through their effect on excitability and threshold; (4) relate changes in threshold to the control of spontaneous uterine activity. The obvious advantage of the method is that, rather than characterizing excitability of a single cell or of several cells by repeated measurements during several hours, it describes the functional state of the entire uterine tissue by a series of rapid measurements. Also, it quantitates threshold after the uterus developed a series of responses provoked by a constant set of decreasing stimuli which stabilizes the organ prior to this critical measurement. The accuracy of the test is increased by repeating it (Fig. 4).

The design of the present studies. Having completed a series of pilot experiments, 48 uterine strips of 24 New Zeland white rabbits were studied systematically. At the twenty-fifth day of pregnancy (P = 9.1 ± 1.2 ng. per milliliter [18]), eight animals were killed and 16 uterine strips examined. Within 12 hours postpartum (P = 0.7 ± 0.2 ng. per milliliter[18]) 12 animals were put to death and 24 uterine strips studied. Postpartum (rather than term pregnant or parturient) uteri were used for studying the condition of high excitability and low threshold because term pregnancy varies with litter

*The significance of differences in W is explained in Comment.

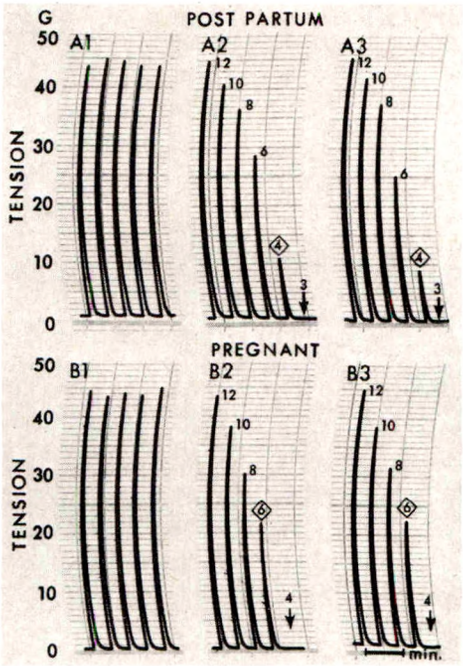


Fig. 5. The effects of pregnancy and parturition on excitability and threshold. Uterus: *A*, approximately 12 hours' postpartum; *B*, 25 days' pregnant. *A1* and *B1*, Maximum WT in steady state (stimulus 12 v per 5 cm.). *A2* and *B2*, The effect of stepwise DEFS. Note that the threshold of postpartum uterus was 4 v. per 5 cm. while that of the pregnant uterus 6 v per 5 cm. Note also that on DEFS WT decreased more rapidly in the pregnant than in the postpartum uterus, indicating suppressed excitability during pregnancy. *A3* and *B3*, The stability of excitability and threshold is illustrated by the similarity in WT responses on the repetition of the experiment.

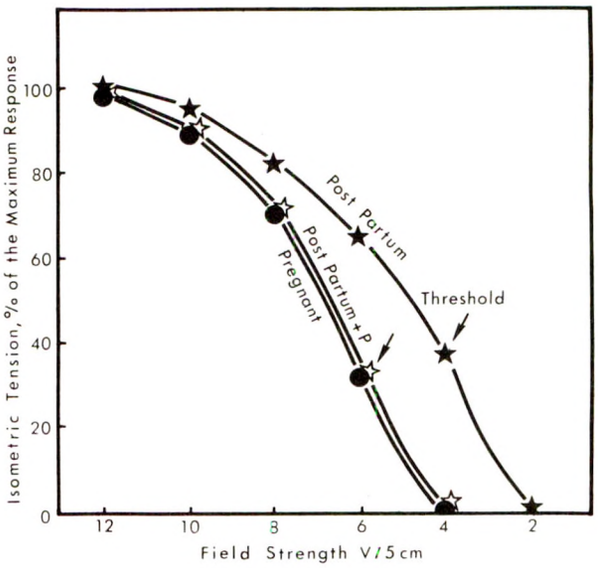


Fig. 6. The effects of pregnancy, parturition, and progesterone treatment on excitability and threshold. A summary of the results obtained in 24 uterine strips approximately 12 hours postpartum, 16 strips 25 days' pregnant, and eight strips postpartum pretreated with P. Note the steeper decrease in WT of the pregnant and postpartum P pretreated than of the postpartum uterine strips during exposure to DEFS. Note also that threshold was higher (6 v. per 5 cm.) in the pregnant and postpartum P pretreated than in the postpartum uteri (4 v. per 5 cm.).

size and parturition usually occurs overnight. The two regulatory conditions, 25 days' pregnant and postpartum, were selected for study to characterize by comparison the effect of progesterone withdrawal (Pw) on uterine excitability and threshold. The premise, that it is Pw (rather than some coincidental change) which lowers threshold and increases excitability, had been examined about 12 hours postpartum in eight uterine strips of four rabbits which received 5 mg. of progesterone (intramuscularly in oil) immediately after delivery.

Results

Excitability and threshold during pregnancy and after birth. Fig. 5 illustrates differences in excitability and threshold between pregnant and postpartum uteri. One uterine strip (*A1*) had been excised approximately 12 hours postpartum (P = 0.7 ± 0.1 ng. per milliliter), while the other (*B1*) was excised on the twenty-fifth day of pregnancy (P = 9.1 ± 1.2 ng. per milliliter). Having built up in both strips maximum WT in a steady state with 12 v. per 5 cm. stimuli (*A1* and *B1*), DEFS was applied: 10, 8, 6, 4, and 3 v. per 5 cm. (*A2* and *B2*). In contrast to the postpartum uterus which responded to stimuli of 4 v. per 5 cm. or higher (*A2*), the pregnant uterus responded only to stimuli of 6 v. per 5 cm. or higher and failed to respond to the 4 v. per 5 cm. field (*B2*). In repeating the study, excitability amd threshold remained unchanged (*A3* and *B3*), illustrating that the differences in excitability and threshold between these two uteri (of different regulatory background) are stable.

Fig. 6 summarizes the results obtained in 12 postpartum, eight pregnant, and four progesterone-treated postpartum uteri. DEFS (from 12 v. per 5 cm. to 10, 8, 6, 4, and 3 v. per 5 cm.) reduced WT step by step in all uteri by activating fewer and fewer cells of the total cell population. Apparently DEFS decreases the ratio: active/inactive cells and reduces WT (through the elastic "yielding" of the inactive cells).[23]

Fig. 6 also illustrates that this decrease in WT resulting from DEFS was steeper in the pregnant and P-treated postpartum uteri than in the untreated postpartum uteri. This finding is evidence that: (1) the excitability of the pregnant (as compared to the

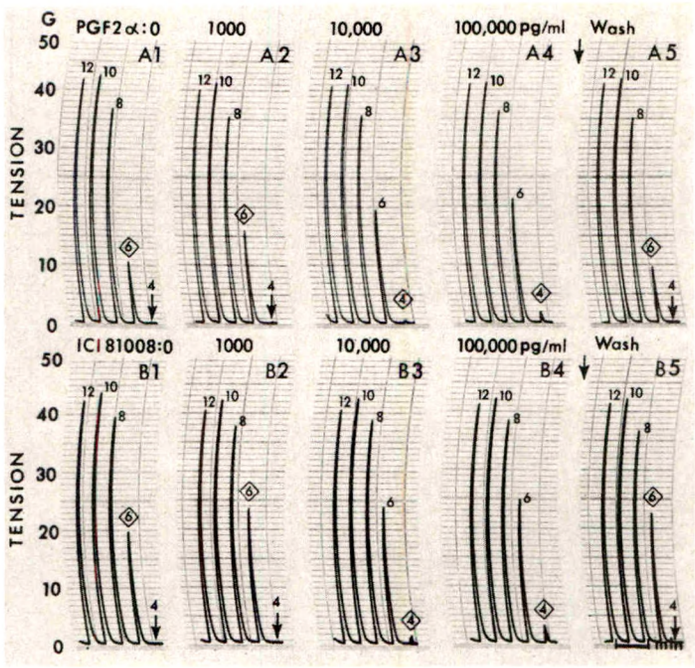


Fig. 7. The effects of $PGF_{2\alpha}$ and ICI 81008 on the excitability and threshold of the pregnant uterus. Uterus: approximately 25 days' pregnant. *A1* and *B1*, Excitability and threshold before treatment with PG's. Threshold was 6 v. per 5 cm. *A2, A3, A4,* and *B2, B3, B4.* The $PGF_{2\alpha}$ and ICI 81008 were administered in concentrations increasing from 1,000 to 100,000 pg. per milliliter. Only when the concentration of $PGF_{2\alpha}$ and ICI 81008 was increased to the excessive value of 100,000 pg. per milliliter (*A4* and *B4*) was the threshold distinctly lowered (from 6 v. per cm. to 4 v. per cm.). The PG-induced increase in WT at higher fields was also moderate. *A5* and *B5,* Washing the tissue with Krebs' solution promptly restored threshold to the pretreatment value (6 v. per 5 cm.).

postpartum) uterus is suppressed; (2) that this effect of pregnancy is due to a P action; (3) P increases while Pw decreases threshold. The difference in threshold between the pregnant (or P-treated postpartum) and the postpartum uteri was very highly significant statistically ($p < 0.001$).

The effects of $PGF_{2\alpha}$ and its analogue ICI 81008 on uterine excitability and threshold. Fig. 7 illustrates that when the pregnant uterus was exposed to increasing concentrations of $PGF_{2\alpha}$ and ICI 81008 the effects of these two compounds on excitability and threshold were similar. However both PG's were effective only at the unphysiologic concentration of about 100,000 pg. per milliliter (*A1, A2, A3, A4,* and *B1, B2, B3, B4*) and even at this high concentration they only lowered threshold from 6 to 4 v. per 5 cm. *(D).* A single washing of the strips with Krebs' solution suspended the effect of both PG's and increased threshold to its original value: 6 v. per 5 cm. *(E).* Apparently, the 25 days' pregnant uterus ($P = 9.1 \pm 1.2$ ng. per milliliter) is insensitive to PG's even at unphysiologic concentration.[2]

Fig. 8 illustrates that, in contrast to the pregnant uterus, the excitability and threshold of the postpartum uterus ($P = 0.7 \pm 0.2$ ng. per milliliter) were strongly affected by $PGF_{2\alpha}$ *(A1, A2, A3,* and *A4)* and ICI 81008 *(B1, B2, B3,* and *B4).* A concentration of $PGF_{2\alpha}$ or ICI 81008 as low as 10 pg. per milliliter lowered threshold from 4 to 3 v. per 5 cm. (*A2* and *B2*) and 1,000 pg. per milliliter reduced it to 2 v. per 5 cm. *(A4* and *B4).* Furthermore, as the PG concentration was increased from 10 pg. per milliliter to 1,000 pg. per milliliter, the same electric field provoked greater and greater WT, demonstrating that by reducing threshold PG's increase excitability. Apparently, the postpartum uterus is highly sensitive to PG's, while the pregnant uterus is insensitive. Fig. 8 also illustrates an additional finding of considerable therapeutic interest. In an earlier study[25] ICI 81008 was about 50 times more efficacious as an abortifacient than $PGF_{2\alpha}$. Since during normal pregnancy PG's cannot display their oxytocic potency, increased efficacy was explained[25] by a prolonged action on microcirculation and fetoplacental or luteal endocrine functions, resulting in Pw and consequent decrease in myometrial threshold and increased excitability. The effects of PG's on microcir-

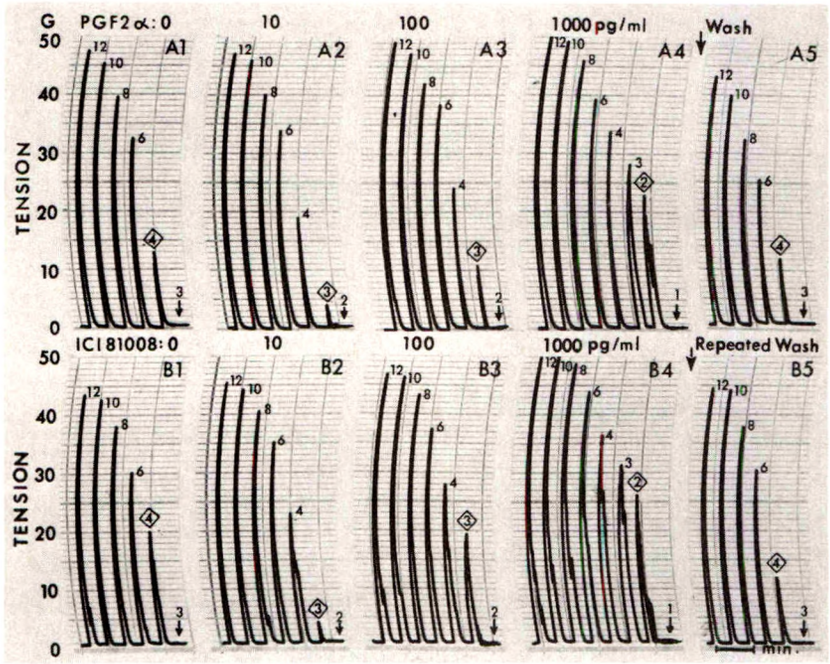


Fig. 8. The effects of $PGF_{2\alpha}$ and ICI 81008 on the excitability and threshold of the postpartum uterus. Uterus: approximately 12 hours; postpartum. *A1* and *B1*, Excitability and threshold before treatments with PG's. In comparison with the pregnant uterus (Fig. 7), threshold was low (only 4 v. per 5 cm.). *A2*, *A3*, *A4*, and *B2*, *B3*, *B4*, $PGF_{2\alpha}$ and ICI 81008 were administered in concentrations increasing from 10 to 1,000 pg. per milliliter. Threshold already decreased (from 4 to 3 v. per 5 cm.) at the PG concentration of 10 pg. per milliliter. As the concentration of PG's was raised, WT (induced by submaximal stimuli) increased, indicating increased excitability. At 1,000 pg. per milliliter PG concentration threshold decreased to 2 v. per 5 cm. *A5* and *B5*, A single wash with Kreb's solution suspended the effect of $PGF_{2\alpha}$ on excitability and threshold, while repeated washing was needed to suspend the effect of ICI 81008, indicating strong binding at target sites.

culation[26] and steroidogenesis[9–16] have already been demonstrated. Fig. 8 illustrates that in comparable doses ICI 81008 (*B1*, *B2*, *B3*, and *B4*) did not reduce threshold or increase excitability more than did $PGF_{2\alpha}$ (*A1*, *A2*, *A3*, and *A4*). This finding indicates that, despite the greater abortifacient efficacy of ICI 81008, the oxytocic potency of these two PG's is similar. However, threshold was restored by a single washing (with Krebs' solution) after $PGF_{2\alpha}$ (*A5*), while repeated washing was needed after ICI 81008 treatment (*B5*). This finding suggests that the binding of ICI 81008 at target sites is stronger than that of $PGF_{2\alpha}$.

Fig. 9 summarizes the results obtained by DEFS in 16 pregnant and 24 postpartum uterine strips. Both PG's affected the postpartum uteri significantly (*A* and *C*), while the pregnant uteri only slightly (*B* and *D*). However, the effects of $PGF_{2\alpha}$ and ICI 81008 on excitability and threshold were similar. Apparently, the high threshold of the pregnant uterus cannot be effectively reduced by either $PGF_{2\alpha}$ or ICI 81008 and thus during normal pregnancy a true oxytocic action cannot be promptly induced by these compounds. This finding exposed the importance of the premise that it is P which sustains high threshold during normal pregnancy and Pw (associated with parturition) which lowers threshold. The following study examined the validity of this premise.

The effect of progesterone on excitability and threshold. Fig. 10 illustrates that twin uterine strips, removed about 12 hours postpartum from a rabbit which when delivered received 5 mg. of P (in oil, intramuscularly), had excitability and threshold indistinguishable from those of 25 days' pregnant uteri (*A1*, *B1*). Also the effects of $PGF_{2\alpha}$ and ICI 81008 on the excitability and threshold of these uteri were characteristic of the 25 days' pregnant uteri (*A2*, *A3*, *A4*, and *B2*, *B3*, *B4*). Since these strips were excised postpartum, approximately 12 hours after the animals were exposed to progesterone treatment, the conclusion is inescapable that P was the regulatory agent which restored low excitability and high threshold (as well as pharmacologic refractoriness) in these uteri.

The relationship between threshold and spontaneous activity and the reversibility of the effects of

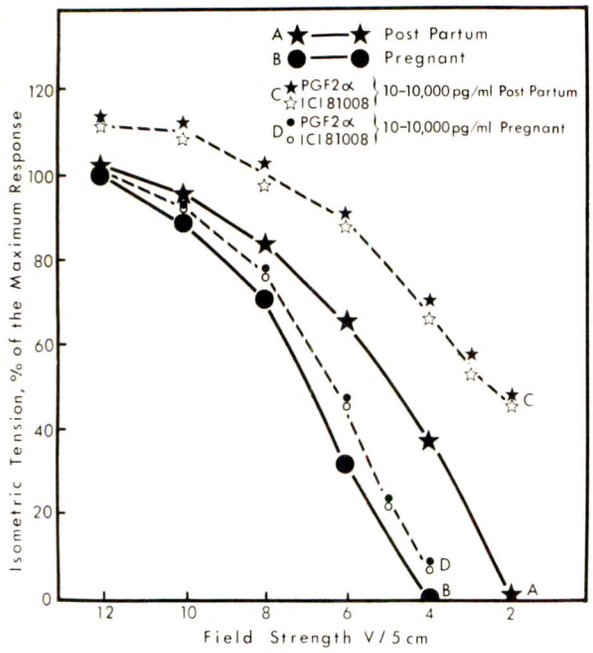


Fig. 9. The effects of $PGF_{2\alpha}$ and ICI 81008 on the excitability and threshold of the pregnant and postpartum uteri. A summary of the results obtained in 16 pregnant and 24 postpartum uterine strips. The WT values were measured within the entire range of 10 to 10,000 pg. per milliliter PG concentrations. However, at the same field strength of stimulation the values were averaged to demonstrate that at all levels of electric stimulation, both PG's significantly increased the excitability of the postpartum uteri (*A* and *C*) in contrast to that of the pregnant uteri (*B* and *D*).

$PGF_{2\alpha}$ and ICI 81008. Fig. 11 illustrates the relationship between threshold and spontaneous activity. After the build up of maximum WT *(A1)*, DEFS revealed that threshold is 4 v. per centimeter and that at this level of threshold there is no spontaneous activity under the present experimental conditions *(A2)*. Treatment of the uterus with 100 pg. per milliliter ICI 81008 promptly triggered spontaneous activity (which increased as the compound penetrated the tissue). WT also increased at all levels of electric stimulation indicating increased excitability as threshold decreased to 1 v. per 5 cm. The first washing did not suspend spontaneous activity *(A3)*, which was explained by the sustenance of low threshold: 2 v. per 5 cm. *(A4)*. Repeating the experiment showed *(A5 to A8)* that a total of six washings were needed to restore threshold to the pretreatment value of 4 v. per 5 cm. and to suspend spontaneous activity. Apparently the magnitude and frequency of spontaneous uterine activity is controlled (at least partly) by changes in threshold. The study also showed that through its effect on threshold ICI 81008 controlled the spontaneous activity of the uterus. Under the present experimental conditions the critical threshold value was ~ 3 v. per 5 cm.; when threshold was below 3 v. per 5 cm. spontaneous uterine activity appeared, while when threshold was above this value it was not generated.

Fig. 11 also illustrates *(A9)* that 100 pg. per milliliter $PGF_{2\alpha}$ also triggered spontaneous activity by increasing excitability and reducing threshold from 4 to 2 v. per 5 cm. However (in contrast to ICI 81008, $PGF_{2\alpha}$ was readily removed as indicated by the disappearance of spontaneous activity, decrease in excitability, and increase in threshold from 2 to 4 v. per 5 cm. by a single washing *(A10)*.

Apparently the effects of both $PGF_{2\alpha}$ and ICI 81008 were reversible. Both compounds were successfully removed from the same uterus by washing with Krebs' solution. However, six consecutive washings were needed to suspend the effect on excitability and threshold of 100 pg. per milliliter ICI 81008 *(A2-A8)*, while a single washing suspended the effect of 100 pg. per milliliter $PGF_{2\alpha}$.

Comment

The present studies were prompted by the recognition that threshold control is a basic regulatory process of considerable interest in reproductive medicine. The therapeutic control of threshold became a pressing clinical problem when it was realized[2] that the pregnant uterus responds only to stimulants when they are given in pharmacologic doses, and even then its response is an unphysiologic contracture. In order to "convert" this contracture response to a normal oxytocic response, for example, high level cyclic IUP (which approximates the maximum working capacity of the organ, IUP = 2WT/R), the uterus had to be *converted* first from a *refractory* to a *reactive* tissue by a marked reduction in P levels.[2–16]

The meaningful examination of threshold control presented technical problems. For, being made up from billions of cells which are significantly different from one another in electrophysiologic properties, even under well-defined regulatory conditions,[23, 24] the threshold of only few cells rather than that of the whole uterus could be characterized with intracellular microelectrodes. Therefore, a technique had to be developed which could quantitate threshold by "averaging" the excitability of all myometrial cells in the excised tissue and in so doing characterize the regulatory condition of the uterus. The in vitro method of "decreasing the electric field stimulation" (DEFS) provided a simple, reliable, and quantitative technique.[2, 17]

This method determined, in approximately 30

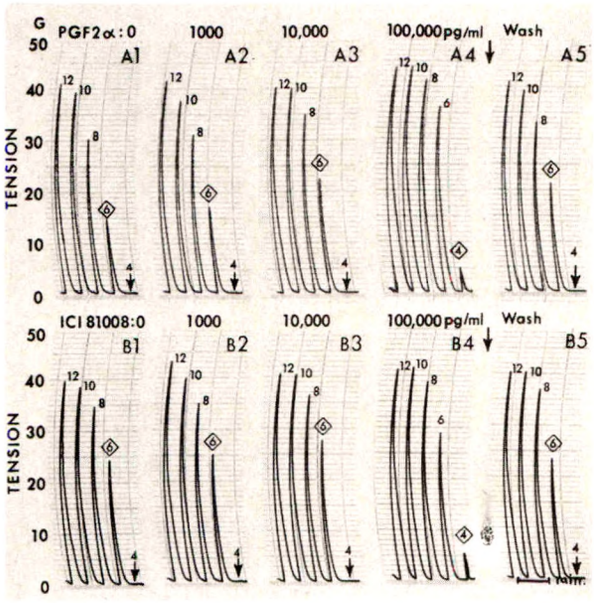


Fig. 10. The effects of $PGF_{2\alpha}$ and ICI 81008 on the excitability and threshold of the progesterone-treated postpartum uterus. Uterus: approximately 12 hours' postpartum, but animal received 5 mg. progesterone after parturition. *A1* and *B1*, Excitability and threshold before PG treatment. Threshold was 4 v. per 5 cm. *A2*, *A3*, *A4*, and *B2*, *B3*, *B4*, $PGF_{2\alpha}$ and ICI 81008 were administered in concentrations increasing from 1,000 to 100,000 pg. per milliliter. *A4* and *B4*, Only when the concentration of $PGF_{2\alpha}$ and ICI 81008 reached the excessive value of 100,000 pg. per milliliter was threshold lowered (from 6 to 4 v. per 5 cm.). The PG-induced increase in WT was also moderate. *A5* and *B5*, Washing the tissue with the Kreb's solution promptly restored threshold to the pretreatment value (6 v. per 5 cm.).

minutes after the exposure of the uterine strips to the in vitro environment: (1) the optimum electric field and maximum WT as reference points; (2) excitability, by changes in WT induced by a standard series of DEFS; (3) threshold, by the minimum field strength which triggered WT; (4) the effect on WT, excitability, and threshold of endogenous regulatory changes; (5) the effect on WT, excitability, and threshold (which together characterize pharmacologic actions) of exogenous stimulants and suppressors; (6) the relationship between threshold and spontaneous activity of the uterus; (7) the relationship between the binding of active compounds and the duration of their effects on WT, excitability, and threshold.

This method (which opened up other lines of investigation as well, for example, in uterine bioenergetics) provides *average* values for uterine excitability, threshold, and pharmacologic reactivity rather than describes single myometrial cells in terms of membrane and action potentials, pacemaker activity, etc.[23, 24, 27] DEFS is no substitute for the intracellular microelectrode technique and vice versa. Therefore, the two procedures should be used side by side for the in depth characterization of uterine function and regulation in those instances when one of them provides partial information only. Nor does DEFS substitute for the study of uterine function in situ. However (as shown by the present experiments), DEFS can characterize the regulatory conditions of the uterus as well as pharmacologic effects, prior to the in situ examination of new therapeutic agents in animal "models" and patients. In some instances DEFS already predicted the outcome of the in situ studies.[28, 29]

At the twenty-fifth day of pregnancy ($P = 9.1 \pm 1.2$ ng. per milliliter) uterine excitability was suppressed and threshold was high (6 v. per 5 cm.). In contrast, postpartum ($P = 0.7 \pm 0.2$ ng. per milliliter) excitability was high and threshold was low (4 v. per 5 cm.). That this significant difference in threshold between pregnant and postpartum uteri ($P < 0.001$) was an effect of P had been demonstrated by restoring high threshold through treatment with P in the postpartum uteri.

It could be argued that excitability and threshold (presently averaged by recording the WT responses of the uterus to DEFS) may not only depend on the excitatory characteristics of the individual myometrial cells of the examined tissue, but also on electric communication between the external and internal layers of these cells. For the electric current applied must penetrate promptly into the stimulated organ to activate its inner layers, or the outer layers must conduct the action potentials rapidly toward the inner layers to promote their participation in activity. Thus radial condition can increasingly affect WT as the stimulus decreases if W is so excessive that the stimulating current cannot penetrate it. This is a valid argument.

However, in the present studies, when 25 days' pregnant and ~ 12 hours[7] postpartum uteri were studied comparatively, W of the postpartum uteri was greater than that of the 25 days' pregnant uteri. If, therefore, radial conduction promoted WT by increasing the participation of the inner layers in activity, then the 25 days pregnant uteri of lesser W were promoted more than the postpartum uteri. Yet the decline of WT on DEFS was steeper and threshold higher in the 25 days' pregnant than in the postpartum uteri. Furthermore, when the animals received P treatment after having given birth, the W of these and the untreated postpartum uteri were similar. Yet the decline in WT of the P-treated postpartum uteri was steeper and their threshold higher than those of the untreated postpar-

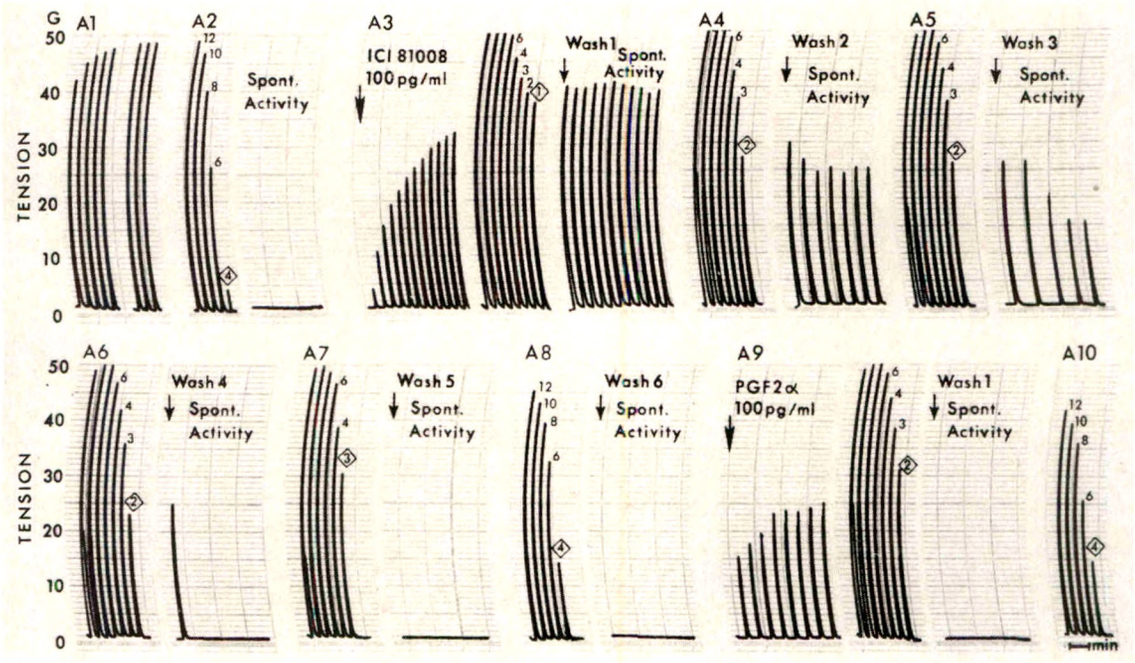


Fig. 11. The effects of ICI 81008 and $PGF_{2\alpha}$ on spontaneous activity, excitability, threshold and recovery. Uterus: approximately 12 hours' postpartum. *A1,* The preparatory steps before the study. *A2,* The effect of DEFS and the assessment of spontaneous activity. When threshold was 4 v. per 5 cm. there was no spontaneous activity (*A3*), but 100 pg. per milliliter of ICI 81008, by lowering threshold (from 4 to 1 v. per 5 cm.), induced it and increased excitability. *A3, A4, A5, A6, A7, A8,* Excitability and spontaneous activity decreased simultaneously when threshold was increased by the removal of ICI 81008 through repeated washing of the uterus. *A9,* The effect of 100 pg. per milliliter $PGF_{2\alpha}$ was similar to that of ICI 81008, but it was suspended by a single washing.

tum uteri. If, therefore, limitations in radial conduction did contribute to the decrease in WT when DEFS approximated threshold values, the 25 days' pregnant uteri of smaller W were affected less by this parameter. Therefore, if the threshold values of the 25 days pregnant and postpartum uteri were corrected for W, the differences between them should increase.

The observed difference in threshold between the pregnant and postpartum uteri is of considerable interest, specifically if the additional finding is considered that, when threshold is high, spontaneous activity is suppressed and the uterus fails in responding to physiologic doses of stimulants. For then it becomes apparent that while the uterus sustains maximum working capacity throughout pregnancy, this potential cannot be converted to high-level cyclic IUP by endogenous or even excessive exogenous stimulation until threshold is lowered by Pw. On the other hand, since the intrinsic uterine stimulant is also present throughout pregnancy, a mere Pw can promote activity by lowering threshold. Thus the present studies fully exposed the significance of endogenous Pw (which occurs near term and precociously in cases of threatened premature labor) and also of Pw exogenously provoked by luteectomy, hypertonic saline, or PG impact.

The present demonstration that in physiologic concentrations $PGF_{2\alpha}$ lowers threshold and increases excitability if threshold has already been lowered by Pw is a point of additional interest. So is the finding that the oxytocic potency of its synthetic analogue ICI 81008 (which is a considerably more potent abortifacient than $PGF_{2\alpha}$) is not greater, only its binding to myometrial sites is stronger and thus its action is prolonged.

Together, the present observations suppliment earlier findings and interpretations, regarding the mechanism of PG action. By showing that oxytocic action is controlled by threshold and threshold by P and that the oxytocic actions of $PGF_{2\alpha}$ and ICI 81008 are similar, the present studies exposed the significance of the effect of PG's on uterine microcirculation, fetoplacental and luteal endocrine functions, and consequently on the regulatory balance of the pregnant uterus. This information may be useful when protocols are designed for the clinical study of PG's.

However, perhaps the broadest and most immediate practical application of the technique of DEFS is in the

reliable screening of new oxytocic compounds and suppressors of therapeutic promise. For, once twin strips are in a steady state (and of course more than one pair may be used simultaneously if desired), the action of a potential uterine stimulant or suppressor can be characterized and quantitated in a few minutes. With the use of, for example, $PGF_{2\alpha}$ (a stimulant) and Naproxen (a suppressor of PG synthesis) as standards, the regulatory potency of new compounds can be characterized comparatively.

As already demonstrated, if the compound is active in vitro,[17] it is also active in situ[23, 25, 28] and the information obtained in animal "models" promotes the design and the predictability of the outcome of clinical trials.[29].

The author is grateful to Drs. A. L. Wapole and M. Dukes of the Imperial Chemical Industries, Limited, for their stimulating comments throughout this study and for the supply of the prostaglandin analogue, ICI 81008; and to Drs. E. M. Southern and G. D. Gutknecht of The Upjohn Co. for useful discussions and for the supply of $PGF_{2\alpha}$. The assistance of Professor M. Pulkkinen and his associates of the Turku University, Finland, in the various phases of this work is acknowledged, and the technical help of Elise F. Csapo, Gabriele Salau, and B. Hayden is appreciated.

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Effect of ampicillin administration on estradiol, estriol, and cortisol levels in maternal plasma and on estriol levels in urine

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Ampicillin treatment in the last trimester of pregnancy had no significant effect on the levels of estradiol, estriol, or cortisol in maternal plasma. Urinary estriol excretion was, however, notably decreased during or following ampicillin treatment in six out of 10 patients. These results suggest that the plasma estrogen assays have greater validity than the urinary estriol assay in assessing fetoplacental endocrine function during a period of ampicillin treatment of the mother.

WE ROUTINELY assess the endocrine function of the fetoplacental unit in high-risk pregnancies by determination of estriol levels in maternal plasma and urine and by determination of estradiol levels in maternal plasma. In view of a recent report that ampicillin administration per se significantly depresses estriol levels in plasma and urine,[1] we wished to determine whether ampicillin exerts a similar effect on plasma estradiol. If it did not, the estradiol assay would be the assay of choice in high-risk pregnancies during ampicillin treatment, especially since we have found that maternal plasma estradiol levels accurately reflect the metabolic status of the fetoplacental unit in such pregnancies.[2]

Cortisol concentrations in the maternal plasma were simultaneously assessed to investigate the possibility that the reported ampicillin effects on estriol levels might be a reflection of maternal stress while treated. Relatively high levels of glucocorticoids in the maternal circulation may result in decreased levels of estrogens in maternal plasma and urine.[3]

Materials and methods

Noninfected patients who were hospitalized in the last trimester of pregnancy for threatened premature labor, mild toxemia, pruritus, or observation for other complications were treated with ampicillin. The steroid assays were performed for 7 consecutive days in most cases. Days 1 and 2 were control, following which a 500 mg. dose of ampicillin was given orally four times per day for 3 days, and days 6 and 7 were post-treatment controls. Any deviation from this schedule is indicated in the results. More than seven determinations of some steroids were performed in some patients and in others less than this amount.

Estriol levels in plasma and urine were measured by radioimmunoassay according to the method of Gurpide and associates.[4] Although this method actually measures total estrogens, estriol generally accounts for at least 80 per cent of estrogens produced in late pregnancy.

Estradiol and cortisol levels in plasma were determined by competitive protein-binding method employing human late pregnancy plasma as the source of the binding proteins, sex hormone–binding protein, and transcortin, as described in detail elsewhere.[5, 6]

Results

All steroid values observed in each patient are shown individually in Figs. 1 to 4.

Some degree of day-to-day fluctuation in plasma steroid levels is generally observed in hospitalized patients and it was observed in the present patients before, during, and after ampicillin treatment. In this context, Figs. 1 to 3 show that ampicillin treatment had no notable effect on the levels of estradiol, estriol, or cortisol in maternal plasma. This was confirmed by

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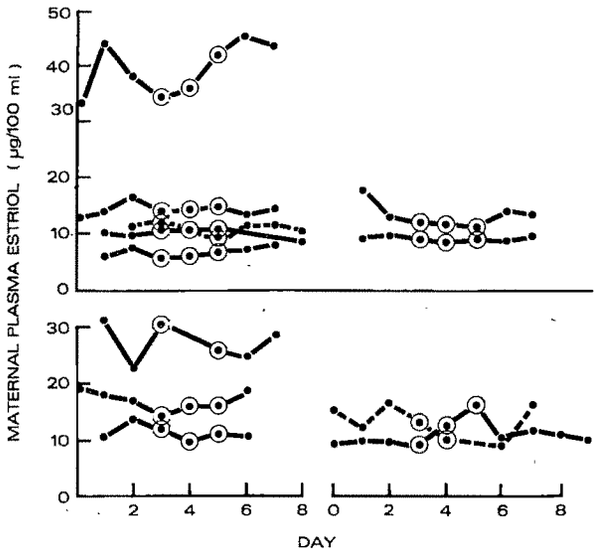


Fig. 1. Maternal plasma estriol concentrations in 11 patients before, during and after ampicillin treatment. Values during treatment are indicated by a dot in a circle.

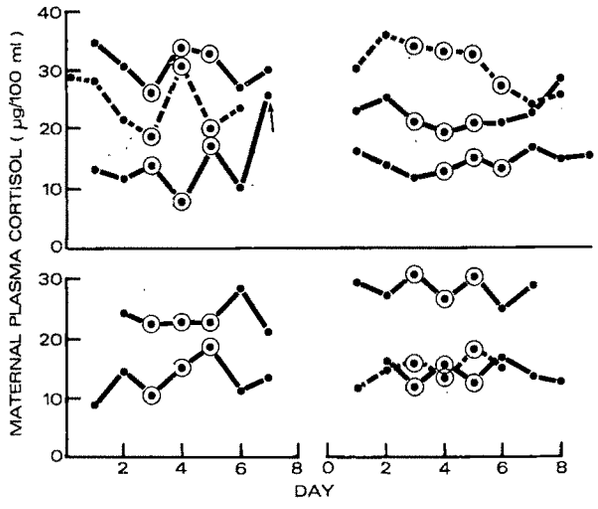


Fig. 2. Maternal plasma cortisol concentrations in 11 patients before, during and after ampicillin treatment. Values during treatment are indicated by a dot in a circle. The small arrow indicates that the patient was in spontaneous labor.

statistical evaluation of data from all patients pooled into before, during, and after ampicillin treatment categories, as indicated in Table I. The differences in mean values in these categories were not significant for plasma estradiol, estriol, or cortisol.

On the other hand, because of the wide range in interpatient values, pooling data for statistical purposes in the case of urinary estriol determinations obscured the fact that ampicillin treatment did affect urinary estriol excretion in some patients, as indicated in Fig. 4.

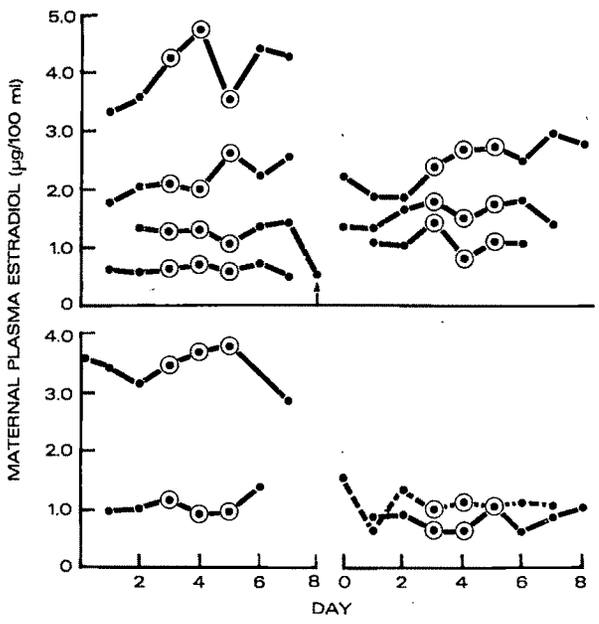


Fig. 3. Maternal plasma estradiol concentrations in 11 patients before, during and after ampicillin treatment. Values during treatment are indicated by a dot in a circle. The small arrow indicates time of onset of heavy bleeding associated with placenta previa.

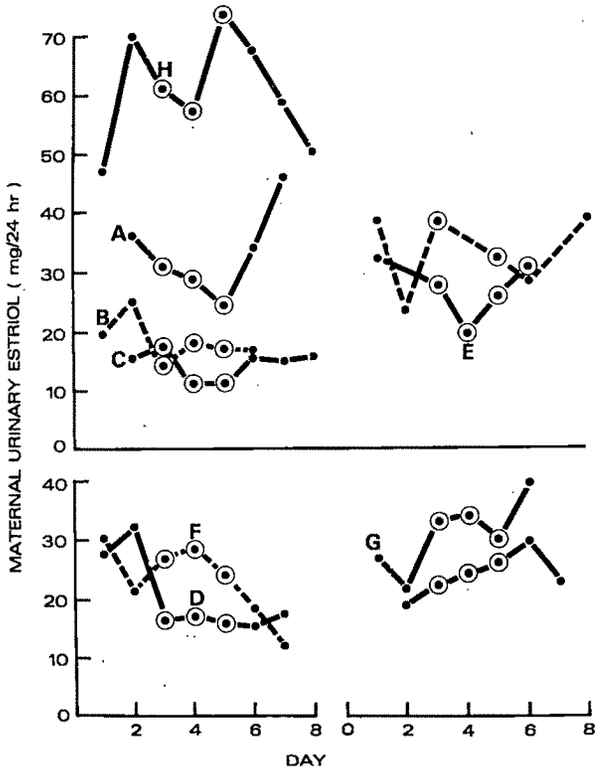


Fig. 4. Maternal urinary estriol concentrations in 10 patients before, during, and after ampicillin treatment. Values observed during treatment are indicated by a dot in a circle.

Table I. Effect of ampicillin treatment on estradiol, estriol, and cortisol levels in maternal plasma and on urinary estriol excretion

Steroid	*Steroid concentrations**		
	Before treatment	*During treatment*	*After treatment*
Plasma estradiol (μg/100 ml.)	1.74 ± 0.18 (26)	1.92 ± 0.20 (32)	1.85 ± 0.23 (22)
Plasma estriol (μg/100 mL)	16.3 ± 2.0 (26)	14.9 ± 1.6 (31)	15.7 ± 2.3 (21)
Plasma cortisol (μg/100 ml.)	21.6 ± 1.7 (23)	21.3 ± 1.3 (34)	20.4 ± 1.4 (22)
Urinary estriol (mg/24 hr.)	28.7 ± 2.7 (19)	29.1 ± 2.3 (32)	31.2 ± 3.5 (19)

*Values are the mean ± standard error; those in parentheses are the number of samples. The differences in mean steroid values observed before, during, and after ampicillin treatment are in each case not statistically significant.

A sustained decrease in values during treatment was observed in four of 10 patients (curves *A* to *D*). A fifth patient (curve *E*) showed an initial decrease in estriol excretion followed by an increase while treated. In another patient (curve *F*) estriol levels decreased markedly following treatment. Values in the four remaining patients, including the two afflicted with pruritus (curves *G* and *H*), were either unchanged or increased during treatment.

Comment

These results indicate that ampicillin treatment in the last trimester of pregnancy has no significant effect on the levels of estradiol, estriol, or cortisol in maternal plasma. The findings with respect to cortisol suggest that maternal stress which could affect fetoplacental function was not a factor in this study; excessive levels of cortisol in the maternal circulation can result in suppression of biosynthesis of estrogen precursors by maternal and fetal adrenals.[3]

An evident decrease in urinary estriol values during ampicillin treatment was observed in about half of the patients. Possibly ampicillin interfered with the enterohepatic circulation of estriol in these particular cases, causing greater excretion in the feces. This effect of ampicillin in pregnant patients has recently been reported by Tikkanen and associates.[7]

Although not all patients were affected (including the two patients with pruritus) the present results tend to agree with the findings of Willman and Pulkkinen[1] in regard to effect of ampicillin treatment on urinary estriol excretion but disagree with the latter authors' findings of a significant concomitant suppression of estriol levels in maternal plasma.

The present results indicate that the plasma estradiol and estriol tests have greater validity than the urinary estriol assay in high-risk pregnant patients during a period of ampicillin treatment.

Two patients were delivered soon after ampicillin treatment. Labor was normal and at term in one case; in the other, an emergency cesarean section was performed for placenta previa with bleeding at 37 weeks' gestation. Of the steroids measured only plasma estradiol reflected this complication. The newborn infants were apparently normal in both cases.

The urinary and plasma estriol determinations were done in the Endocrine Laboratory of the Royal Victoria Hospital. The authors thank Doctors A. DeLeon and J. Chow for assistance.

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Human pregnancy-associated plasma proteins during the postpartum period

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The plasma levels of four pregnancy-associated plasma proteins (PAPP's) and pregnancy zone protein (PZP) were studied in women after delivery of a single viable infant by gel immunodiffussion methods. Data from 89 random samples and 85 serial specimens from five women revealed that both PAPP-B and HPL (PAPP-D) disappeared within a day after delivery. PAPP-A showed a rapid drop in the first 2 or 3 days post partum and became nondetectable in 4 to 6 weeks, with a half-life of 3 to 4 days. PAPP-C had a sharp decrease by the second postpartum day and was not detected 3 to 4 weeks later, with a half-life of 1 to 2 days. None of the PAPP's was detected again during the rest of the 14 postpartum weeks studied. In contrast, the PZP showed a much slower decrease or even a temporary increase during the first 2 weeks post partum; it remained readily detectable over the entire 14 weeks studied.

CERTAIN proteins become readily detectable in maternal plasma during gestation that are undectectable prior to pregnancy.[1–5] Four such pregnancy-associated or -specific plasma proteins (PAPP's) were revealed with immunologic methods in our laboratory.[6–8] They differed from each other in molecular weight, sedimentation rate, electrophoretic mobility, isoelectric point, as well as other properties,[7] and were not detected in cord serum. Three of them have been purified over 100-fold and monospecific antisera against them have been prepared.[9] By comparing the immunologic specificity of monospecific antisera to the PAPP's and possibly related proteins from other investigators,[9] it was revealed that one of the PAPP's (PAPP-D) is human placental lactogen (HPL). Another (PAPP-C) is identical to the pregnancy-specific β_1-glycoprotein (SP_1) described in 1971 by Bohn.[10] The other two (PAPP-A and PAPP-B) may represent newly discovered human plasma proteins since they were unrelated to many of the proteins which had been described in association with pregnancy, such as α_1-fetoprotein, oxytocinase, chorionic gonadotropin, pregnancy zone,[11] α_2-pregnoglobulin,[12] pregnancy-associated globulin (PAG),[13] Pal,[14] new serum α-macroglobulin,[15] Xh,[17] SP_3,[10] and pregnancy-associated α_2-globulin.[18] Evidence has been obtained which indicates that the latter eight actually represent the same protein.[19] For convenience and historical reasons, this will be referred to below as pregnancy zone protein (PZP).

During gestation, the plasma levels of these PAPP's rose steadily during the second trimester and increased steeply in the early part of the third trimester.[20] PAPP-A continued to rise until the end of gestation, whereas PAPP-C reached a plateau late in the third trimester. HPL (PAPP-D) showed a trend similar to PAPP-C. The quantitative pattern of PAPP-B could not be determined because of technical reasons, but it was detected with increased frequency as gestation progressed. The present report is concerned with measurement of the PAPP's, as well as the PZP in the plasma of women during the postpartum period.

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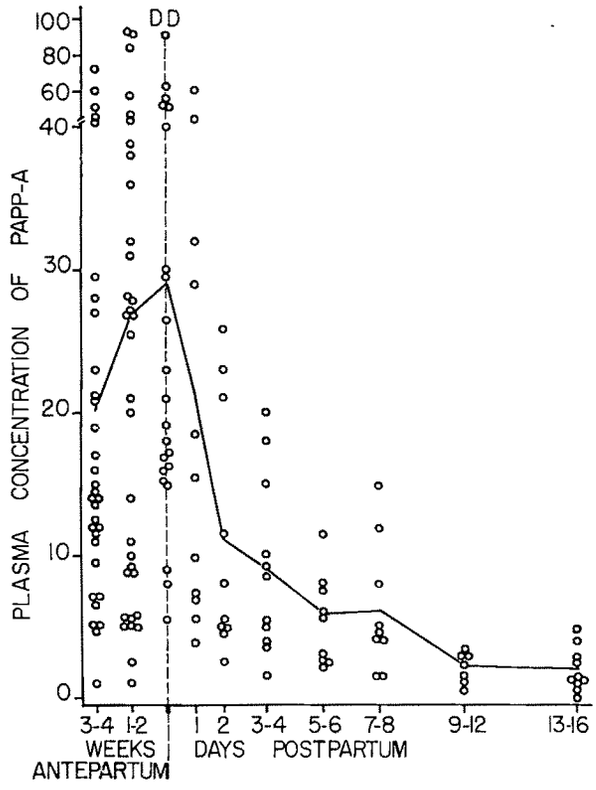


Fig. 1. Scatter diagram of PAPP-A concentration in early postpartum plasma. Means are connected with a solid line. *DD,* day of delivery.

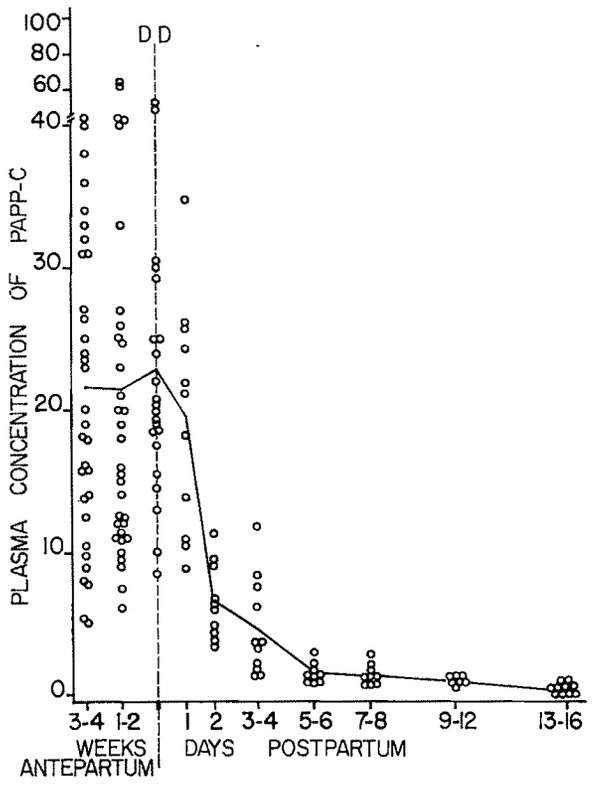


Fig. 2. Scatter diagram of PAPP-C concentration in early postpartum plasma. Means are connected with a solid line. *DD,* day of delivery.

Materials and methods

Two collections of plasma samples were obtained from the University of Miami Medical Center. The first consisted of 89 specimens from 55 women, representing single or repeat bleedings. They were obtained 0 to 16 days after term delivery of a single viable baby. These samples were assayed in duplicate for the concentration of PAPP's by the quantitative crossed-immunoelectrophoresis method described earlier.[20] The second group of 85 plasma samples were drawn serially from five normal women during the period between 6 weeks ante partum* and 14 weeks post partum. None of these received estrogen therapy during the first 4 weeks after delivery; one patient started estrogen-progesterone contraception (Norinyl) 8 weeks post partum. The concentrations of the PAPP's, as well as PZP, were measured in duplicate by Laurell's[21] "rocket" electroimmunodiffusion method with the monospecific antisera available, as well as by crossed-immunoelectrophoresis (for the detection of PAPP-B).[20] The former technique is the same as the latter, except that electrophoresis in the first direction is omitted. The values obtained by both methods showed good agreement in the case of PAPP-A, -C, and -D.

Poly- and monospecific rabbit antisera to the PAPP's for the assay system were prepared as described elsewhere.[9] Goat monospecific antiserum to PZP was generously donated by Dr. Berne of Indiana University.

A reference pregnancy plasma pool was concentrated fourfold by lyophilization to serve as a standard, and its contents of PAPP and PZP were arbitrarily assigned values of 100 units per milliliter, as described earlier.[20]

Results

Random samples. The data from these samples were grouped according to the time post partum, and charted in relation to the values previously obtained during late pregnancy.[20] As shown in the scatter diagram of Fig. 1, the plasma concentrations of PAPP-A increased until the day of delivery, then

*In this report, ante partum refers to the time before delivery.

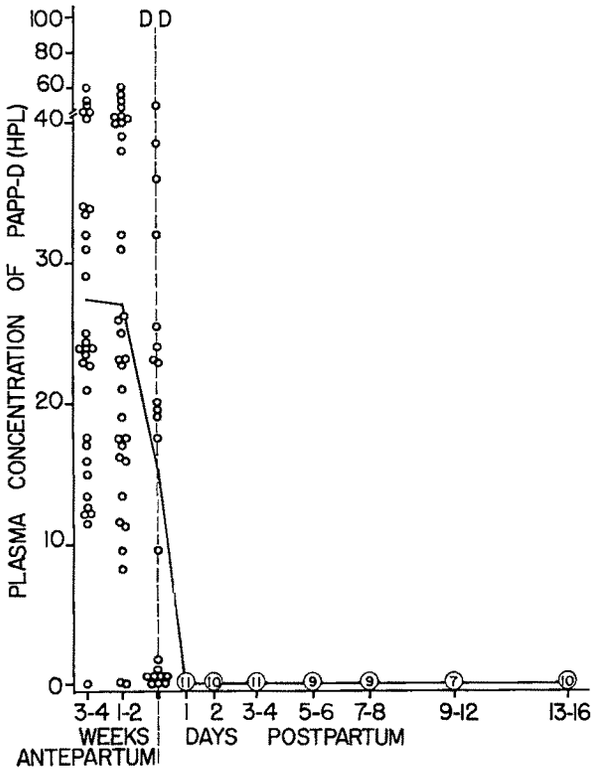


Fig. 3. Scatter diagram of HPL (PAPP-D) concentration in early postpartum plasma. Means are connected with a solid line. *DD,* day of delivery. Numbers in circles refer to the number of samples tested.

Table I. Detection of PAPP-B in the postpartum plasma samples by crossed-immunoelectrophoresis

Time of pregnancy	*No. with PAPP-B/ No. examined*	*Per cent*
Last month of pregnancy	32/65	49
Day of childbirth	11/22	50
1 to 16 days postpartum	0/67	0

showed a sharp drop in the first 2 days post partum, followed by a slower decrease to low levels during the rest of the period studied. It remained detectable in almost all specimens at the end of the second week post partum. The mean PAPP-A levels at 1 and 2 days post partum were 70 and 40 per cent of that found on the day of delivery, suggesting a half-life of 1 to 2 days for this pregnancy protein.

The PAPP-C levels 1 day after delivery were not much different from those seen in the last month of pregnancy. By the second day post partum, however, a sharp drop of more than 50 per cent occurred, which was followed by a more gradual decrease over the next 2 weeks (Fig. 2). By the end of the second week, PAPP-C was nondetectable in five of the 10 specimens tested. Both PAPP-B and HPL (PAPP-D) started to disappear rapidly on the day of childbirth and were not detectable 1 day after delivery or thereafter (Fig. 3 and Table I).

The rise in the PAPP's during pregnancy and their fall after delivery are diagrammatically summarized in Fig. 4, which incorporates the above data with those obtained earlier.[20] The median values, although often slightly lower, showed similar trends. PAPP-A was the last to increase during gestation, but it continued to rise until the day of delivery. PAPP-C began to rise earlier and less steeply, but reached a plateau during the last several weeks of pregnancy. Both PAPP-A and PAPP-C dropped sharply after delivery, but PAPP-C decreased more rapidly in the first week of the postpartum period. HPL (PAPP-D) rose steeply during the third trimester, reached its highest concentration during the middle part of the third trimester, and then dropped precipitously after delivery to nondetectable levels. Although PAPP-B cannot yet be quantitated because of technical reasons, it also appeared to increase during the last trimester, but like HPL (PAPP-D) it became nondetectable very rapidly after childbirth.

Serial sampling of individual women. Because of the rather large variation between women in the plasma concentrations of PAPP's shown above (Figs. 1 to 3) and in earlier studies,[20] it was felt of importance to serially determine the PAPP concentrations in individual women shortly before delivery and through the postpartum period. For this reason, serial samples from five patients were analyzed approximately biweekly during 6 weeks ante partum, daily during the first week post partum, and then weekly for an additional 13 weeks. The PZP was also analyzed in these specimens to determine if it correlated with any of the PAPP's.

Although the levels of these proteins showed the expected wide range of concentrations in the different women, in the same individual they did not show wide fluctuations, but revealed smoothly progressive changes over these time periods (Fig. 5). In the three instances where samples were available on the day of delivery and at 1 day post partum, both PAPP-A and PAPP-C showed a significant drop in the first day. HPL (PAPP-D) became nondetectable in all instances at 1 day post partum, as did PAPP-B. In all five patients, PAPP-A and PAPP-C showed a pattern of rapidly decreasing levels during the first 2 weeks, similar to that seen with the random samples. PAPP-C became uniformly nondetectable by 3 or 4 weeks; PAPP-A dropped to trace levels at 3 to 5 weeks and was no

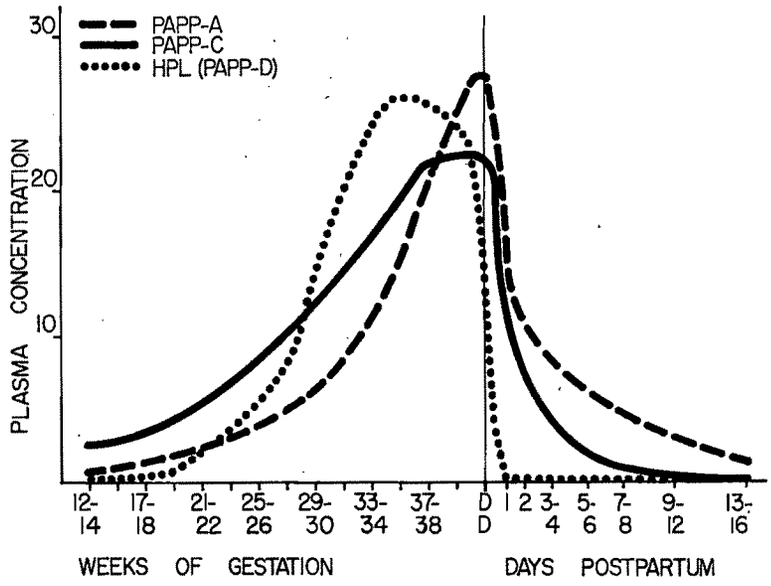


Fig. 4. Mean concentration of PAPP-A, PAPP-C, and HPL (PAPP-D) in random plasma samples during gestation and after delivery. *DD*, day of delivery.

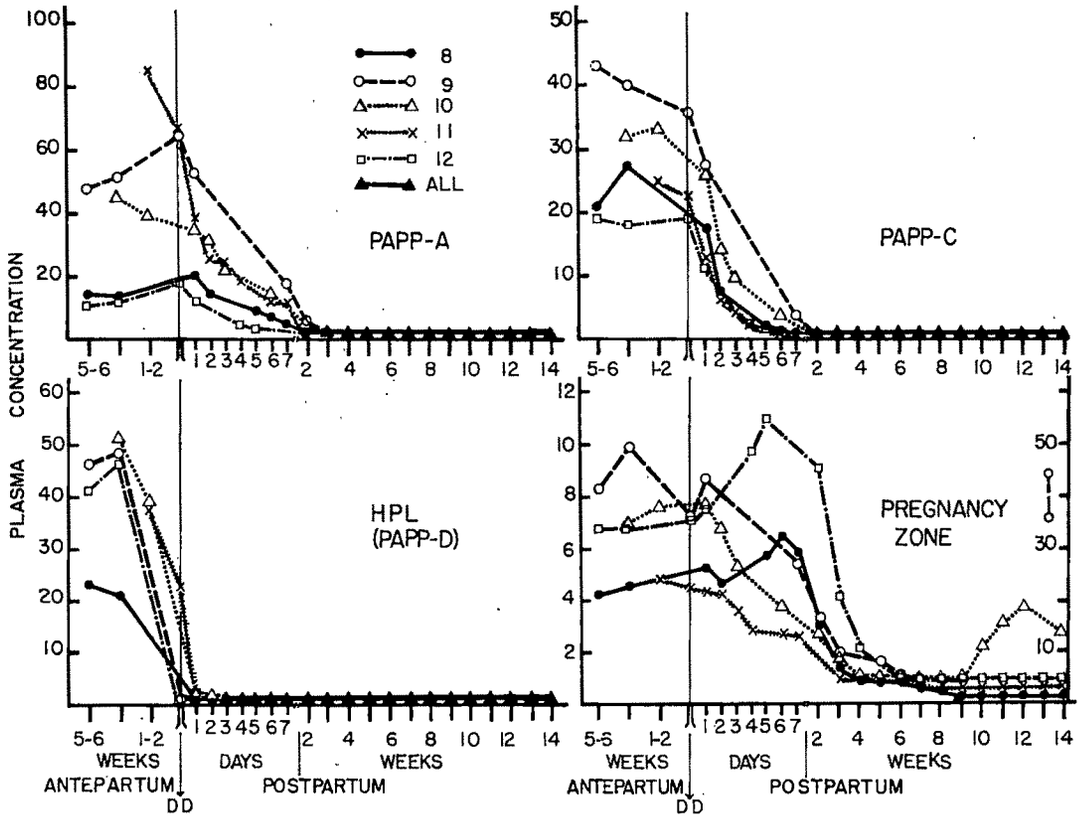


Fig. 5. Plasma concentration of PAPP-A, PAPP-C, HPL (PAPP-D), and pregnancy zone protein in serial samples from five women before and after delivery. *DD*, day of delivery.

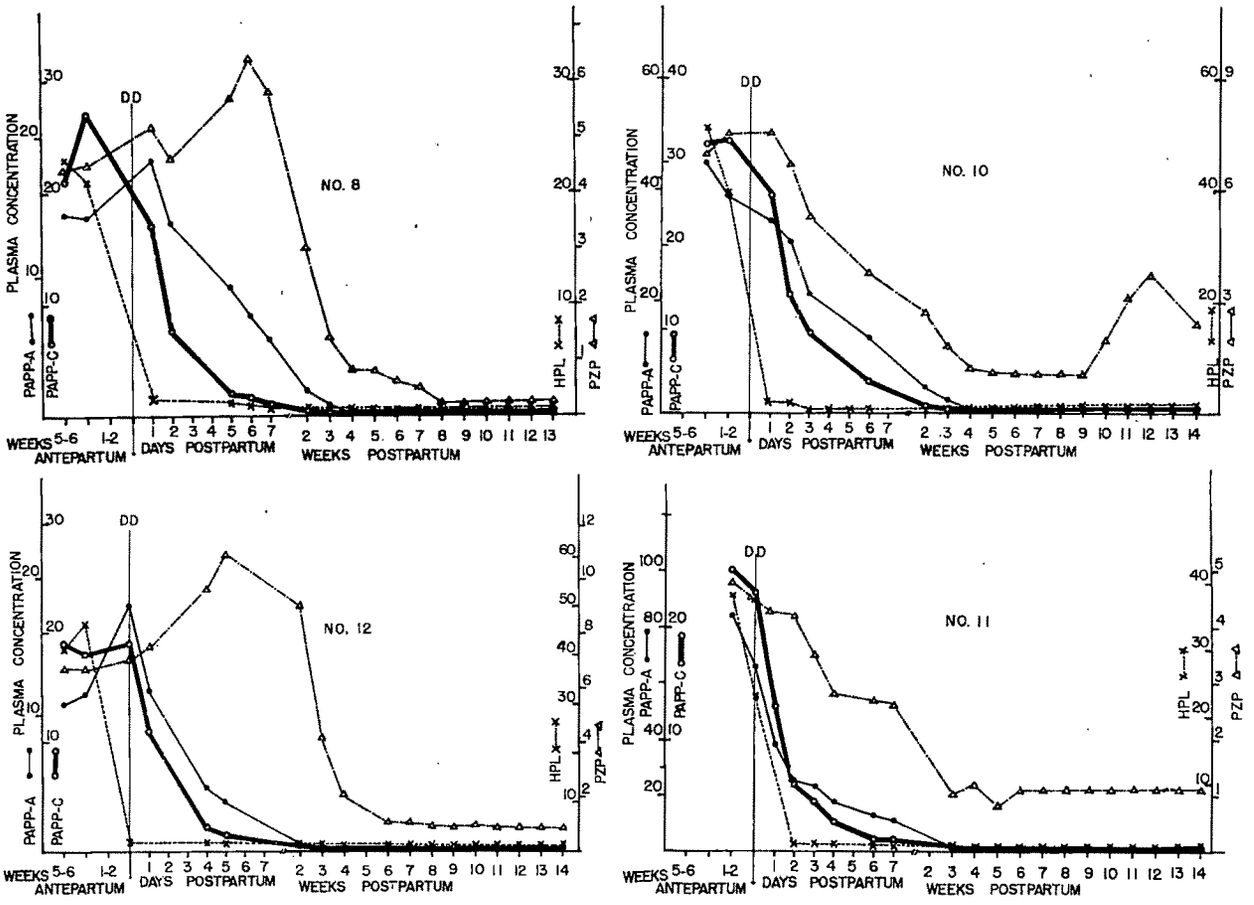


Fig. 6. Comparison of changes in plasma concentration among PAPP-A, PAPP-C, HPL (PAPP-D), and pregnancy zone protein in four women after delivery. The trends of these proteins in the fifth woman (No. 9) were similar to those in patient No. 11. *DD,* day of delivery.

longer detected in any case after 6 weeks. On the other hand, the PZP did not show any striking changes in concentration until the second and third weeks after delivery. Its level in two women (Nos. 8 and 12) even showed a temporary increase during the first week. The rate of decrease of PZP was also significantly slower than that seen with the PAPP's. In further sharp contrast to the PAPP's, PZP remained detectable by these methods during the entire period studied, up to 14 weeks post partum. A small rise in PZP was observed in one women (No. 10) during the period between the tenth and fourteenth weeks post partum, coinciding with the start of hormonal contraception. In four of the five women studied, the concentration of PZP was relatively low just prior to delivery, in relation to the reference specimen which represented a fourfold concentrate of a late pregnancy plasma pool.

The rates of change in the various PAPP levels and PZP in single individuals are shown in Fig. 6. It appeared that the decrease and disappearance of the PAPP's were sequential in all of the five women, starting with HPL (PAPP-D) and PAPP-B, followed by PAPP-C, and then PAPP-A. The time course of the changes in PZP concentrations showed no correlation with the rate of decrease of any particular PAPP in these individuals. By comparing the plasma levels in three women (Nos. 8, 10, and 11) during the first few days post partum, the half-lives of PAPP-A and PAPP-C were roughly estimated to be 3 to 4 and 1 to 2 days, respectively, the former being slightly longer than the value obtained from the random samples.

Comment

The rapid decrease and disappearance of all the PAPP's was shown to coincide crisply with the termination of gestation. Furthermore, none of the PAPP's increased or reappeared after becoming nondetectable during the 3 month post partum period. Bohn[10] also failed to detect pregnancy-specific β_1-glycoprotein (which is identical to PAPP-C) in any of the three serum samples collected 5 to 14 weeks after delivery. In sharp contrast, the PZP remained at relatively high

levels for the first 2 weeks post partum, although it showed a gradual decrease during this period. The PZP was still present in readily detectable concentrations during the rest of the 14 weeks studied. These latter data are in general agreement with the findings of Studd and associates,[4] Bohn,[10] Berne,[12, 22] and von Schoultz,[23] who detected PZP in women 2 to 3 months after delivery of normal infants. These findings suggest that the PAPP's are direct or primary responses to gestation, while the PZP is secondarily related to pregnancy. The elevation of PZP in patients with various malignancies is compatible with this concept, [12, 15] in contrast to the finding that none of the PAPP's has thus far been seen in the serum of cancer patients.[24] In any event, it is reasonable to assume that the PZP differs from all the PAPP's with regard to their biologic activities.

The smooth progression of PAPP levels in individual patients during the postpartum period suggests that the wide range of values observed in different women at the same stage of the gestational process is valid. This broad scatter of values indicates that any potential diagnostic usefulness of these measurements will require serial samples, or very extreme divergence from the normal range. It would be of interest to study the PAPP levels in individual women during several pregnancies to determine if a tendency toward low or high concentrations during gestation is characteristic of that individual.

Because the PAPP's do disappear so rapidly following delivery, it is reasonable to speculate that they may arise from the placenta or fetus. Since cord blood and amniotic fluid do not contain any PAPP's detectable with the technique used, the fetus is unlikely to be the source. Quantitative studies are currently being carried out to determine if the placenta synthesizes PAPP-A, -B, and -C, in addition to HPL.

The half-life of HPL (PAPP-D) has been shown to be 20 to 30 minutes.[25, 26] The present study confirmed the fast rate of disappearance of this hormone, which became undetectable by the methods used 1 day after the removal of the tissue of origin. The rapid disappearance of PAPP-B during the postpartum period suggests that it also may have a very short half-life. The half lives of PAPP-A and PAPP-C, as estimated with random samples, were similar to—but somewhat shorter than—those with serial samples. Because of the large variation in the PAPP levels among individuals, the estimates from serial samples may be more precise, although a much larger series will be required for an accurate determination. In addition, samples collected at closer time intervals just prior to delivery will be required for careful analysis of the changing PAPP levels during this critical period.

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The association between abnormal glucose tolerance (hyperglycemia and hypoglycemia) and estriol excretion in pregnancy

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In 2,000 consecutive patients having glucose tolerance tests in pregnancy hyperglycemia (≥ ninety-fifth percentile) was associated with increased placental weight ($p < 0.01$) but not with increased fetal birth weight. Patients with hypoglycemia (≤ fifth percentile) were more likely to have small-for-dates babies ($p < 0.01$). Perinatal death was related to maternal glucose tolerance, being reduced from 1.3 per cent in the total series to 0.6 per cent when normoglycemia was present ($p < 0.05$); it was significantly increased in the presence of maternal hyperglycemia ($p < 0.001$) and hypoglycemia ($p < 0.01$). A combination of abnormal glucose tolerance and subnormal estriol excretion detected pregnancies with significantly higher incidences of fetal and placental growth retardation, major fetal malformations, and perinatal deaths. Moreover, the combination of normoglycemia and normal estriol excretion (62.3 per cent of patients) was associated with a very favorable pregnancy outcome (0.4 per cent perinatal death rate). Hypoglycemia was at least as significant as hyperglycemia in terms of unfavorable pregnancy outcome, especially when associated with subnormal estriol excretion.

GLUCOSE TOLERANCE testing in pregnancy has principally been directed toward the detection of diabetes mellitus.[1] Because the glucose tolerance test has been regarded as an unsuitable laboratory investigation for routine performance in all pregnancies,[2] a policy of undertaking the test only in selected patients has led to publication of many means of selection of possibly diabetic patients.[3, 4] However, it is acknowledged that a number of patients with gestational diabetes will still be missed by such methods of selection.[4]

If the glucose tolerance test was more broadly interpreted and detected associations with pregnancy complications, then routine performance of the test in all pregnant patients may be justified. In the process all patients with gestational diabetes would be diagnosed.

Diabetes mellitus in pregnancy has an increased incidence of low estriol excretion,[5] while subnormal urinary estriol excretion in pregnancy is associated with an increased incidence of small-for-dates babies and placentas, perinatal death, and fetal malformations.[6, 7]

The purpose of the present study was to assess a new method of classification of glucose tolerance test results in pregnancy by defining hyperglycemia, hypoglycemia, and normoglycemia, and to evaluate the significance of urinary estriol excretion when glucose tolerance was abnormal in late pregnancy. The pregnancy outcome in each classification when the tests were performed in a consecutive series of patients is reported.

Materials and methods

Both glucose tolerance tests and urinary estriol excretion are routinely measured on all prenatal

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patients at the Mercy Maternity Hospital. This series consisted of 2,000 consecutive patients, 1,000 clinic, and 1,000 private. The only patients excluded from analysis were 9 known diabetics, all of whose infants survived, and those with multiple pregnancies.

A standard 50 Gm. oral glucose tolerance test was performed at 32 to 34 weeks' gestation in the morning following an overnight fast. Plasma glucose was measured in capillary blood, obtained from a finger prick, by the glucose oxidase method using a Beckman glucose analyzer. Plasma glucose was measured on a fasting specimen and 1, 2, and 3 hours after the ingestion of glucose.

The ninety-fifth and fifth percentiles were calculated for each plasma glucose reading. These were separately evaluated for the clinic and private groups of patients,[8, 9] and are shown in Table I.

Hyperglycemia was diagnosed when the plasma glucose equalled or exceeded the ninety-fifth percentile on any of the four plasma glucose measurements. Hypoglycemia was diagnosed when the plasma glucose equalled or was less than any of the fifth percentiles. Normoglycemia was defined as being present when all four glucose levels during the tolerance test fell within the ninety-fifth to fifth percentile ranges.

Urinary estriol excretion was measured in all patients on one or more occasions from 30 to 34 weeks' gestation, the total number of assays performed in this study being 6,078. This was measured with the method of Brown and associates[10] and was regarded as subnormal when one or more values were obtained below a line joining 8 mg. per 24 hours at 30 weeks' gestation and 12 mg. per 24 hours at 40 weeks' gestation and thereafter.[11]

Large-for-dates infants were diagnosed when birth weight was above the ninetieth percentile and small-for-dates infants when below the tenth percentile according to gestational age for infants born in this community.[12] The ninetieth and tenth percentiles for defining large and small-for-dates placentas were those reported by Ratten and associates.[13]

A fetal malformation was regarded as major if it was of such severity that it interfered with the function of all or part of the infant. Perinatal death included any stillbirth after the twentieth week of pregnancy and deaths occurring in liveborn infants within 28 days of birth.

In this prospective series management of the pregnancy was unaffected by the finding of an abnormal glucose tolerance with the exception of those results indicative of gestational diabetes (maximum plasma glucose level of 180 mg. per 100 ml. or above and 2 hour level of 140 mg. or above).

Table I. Ninety-fifth and fifth percentiles for clinic and private patients

Glucose tolerance test	*Plasma glucose percentiles (mg./100 ml.)*	
	Ninety-fifth	*Fifth*
Fasting:		
Clinic	93	61
Private	91	65
1 Hour:		
Clinic	165	88
Private	172	95
2 Hours:		
Clinic	128	68
Private	132	74
3 Hours:		
Clinic	103	55
Private	106	56

Results

The 2,000 pregnancies were assessed for the incidences of low urinary estriol excretion, abnormalities of birth weight and placental weight, major fetal malformations, and perinatal death. The over-all results for the 2,000 patients are shown in Table II as well as the pregnancy outcome when evaluated according to whether urinary estriol excretion was normal or low.

Table III shows the pregnancy outcome according to the pattern of glucose tolerance (hyperglycemia, normoglycemia, or hypoglycemia) and according to whether estriol excretion was normal or subnormal.

Twenty-seven patients had both a hyperglycemic and a hypoglycemic level during the glucose tolerance test, and five of these (18.5 per cent) had subnormal estriol excretion. These patients are included in the results of both types of abnormal glucose tolerance. There were 14 tests diagnostic of gestational diabetes and in these patients there was one perinatal death, which was due to major malformations.

Comment

This classification of glucose tolerance tests in pregnancy has defined two types of abnormality—hyperglycemia and hypoglycemia—leaving a group properly defined as normal—normoglycemia.

The various definitions of gestational diabetes[14] attest to the confusion in what may be regarded as an abnormal glucose tolerance test. The concept of hyperglycemia and hypoglycemia in pregnancy has not been previously evaluated and was pursued to determine whether these were associated with pregnancy complications. The outcome of the pregnancies in those patients with abnormal glucose tolerance was

Table II. Pregnancy outcome according to urinary estriol excretion

Estriol excretion	*Patients*		*Pregnancy complications (per cent)*					
	No.	*Per cent*	*Small-for-dates baby*	*Large-for-dates baby*	*Small-for-dates placenta*	*Large-for-dates placenta*	*Major malformations*	*Perinatal deaths*
Total series	2,000	100.0	9.8	9.2	11.0	9.7	3.8	1.3
Normal estriol	1,805	90.2	7.9	9.9	9.5	9.9	3.5	0.9
Subnormal estriol	195	9.8	27.7	2.6	25.1	8.2	6.7	5.1

Table III. Pregnancy complications according to the type of glucose tolerance and urinary estriol excretion

Glucose tolerance and estriol excretion	*Patients*		*Pregnancy complications (per cent)*					
	No.	*per cent*	*Small-for-dates baby*	*Large-for-dates baby*	*Small-for dates placenta*	*Large-for-dates placenta*	*Major malformations*	*Perinatal deaths*
Total series	2,000	100.0	9.8	9.2	11.0	9.7	3.8	1.3
Hyperglycemia:								
Total	315		9.5	9.5	9.8	14.6	2.9	3.2
Normal estriol	278	88.3	7.6	10.4	8.6	15.1	2.2	2.5
Subnormal estriol	37	11.7	24.3	2.7	18.9	10.8	8.1	8.1
Normoglycemia:								
Total	1,366		8.5	9.3	10.8	8.6	3.9	0.6
Normal estriol	1,245	91.1	6.8	9.9	9.4	8.8	3.7	0.4
Subnormal estriol	121	8.9	25.6	3.3	24.8	6.6	5.8	2.5
Hypoglycemia:								
Total	346		15.6	7.8	13.6	9.8	4.3	2.6
Normal estriol	304	87.9	12.5	8.9	10.9	9.9	3.6	1.6
Subnormal estriol	42	12.1	38.1	0	33.3	9.5	9.5	9.5

compared with that in the normoglycemia group.

Low urinary estriol excretion was seen to select pregnancies with a greatly increased incidence of small-for-dates babies ($p < 0.001$) and placentas ($p < 0.001$), with a reciprocal reduction in the incidence of large-for-dates babies ($p < 0.001$), but little alteration in the incidence of large-for-dates placentas (Table II). Furthermore, there was a doubled incidence of major malformations ($p < 0.05$) and a sixfold increase in perinatal mortality rate ($p < 0.001$) in pregnancies where estriol excretion was subnormal. This confirms the value of this investigation in prediction of pregnancy complications. These associations were seen to remain whether glucose tolerance was normal or abnormal (hyperglycemia or hypoglycemia) (Table III).

Glucose is an essential metabolite for fetal development and growth and presumably exerts much of its influence upon fetal growth via the insulin levels in the fetus responding to the amount of glucose transferring across the placenta. Although the placenta may prevent unlimited transfer of glucose to the fetus,[15] fetal glucose levels will qualitatively mimic those in the mother. In the presence of maternal hyperglycemia the increased levels of glucose transported to the fetus appeared to stimulate placental growth ($p < 0.01$) before fetal size was excessively stimulated ($p > 0.05$) (Table III). Placental hypertrophy, a characteristic of diabetes in pregnancy, is apparently an earlier effect of hyperglycemia than that of fetal macrosomia.[16] Karlsson and Kjellmer[17] were unable to show any relationship between the degree of hyperglycemia in the mother and the incidence of infants overweight at birth in their series of controlled diabetic pregnancies. However, relative glucose deprivation of the fetus, as may occur when there is maternal hypoglycemia, appeared to exert its influence principally by retarding intrauterine growth of the fetus (8.5 per cent in the normoglycemia group and 15.6 per cent in the hypoglycemia group were small for dates—Table III) ($p < 0.01$). With the ability of low urinary estriol excretion to detect intrauterine growth retardation, the combined application of both glucose tolerance testing and urinary estriol estimation selected groups of pregnancies where the incidence of small-for-dates babies ranged from 6.8 per cent when both tests were normal, to 38.1 per cent when estriol excretion was low in the presence of hypoglycemia (Table IV).

The association between hypoglycemia and small-for-dates babies and placentas was markedly increased when estriol excretion was low ($p < 0.001$ and $p < 0.001$, respectively). Moreover, there were no large-for-dates babies when estriol excretion was low in the presence of hypoglycemia (Table III).

Fetal malformations have been shown to be more common in pregnancies where estriol excretion is low, although with the exception of anencephaly or primary adrenal hypoplasia, this association is not causal but due to the high incidences of fetal growth retardation.[7] Although the incidence of major fetal malformations did not appear to be influenced by maternal glucose tolerance ($p > 0.05$ for both hyperglycemia and hypoglycemia), a trend toward a high incidence of malformations was noted when estriol excretion was low (Table III). This suggested that low estriol excretion was more selective for fetal malformations when seen in association with abnormal glucose tolerance.

Perinatal mortality rate was related to maternal glucose tolerance, being reduced from 1.3 per cent in the total series to 0.6 per cent when normoglycemia was present ($p < 0.05$). This was significantly increased in maternal hyperglycemia ($p < 0.001$), and hypoglycemia ($p < 0.01$) (Table III). The cause of fetal death in maternal diabetes mellitus is, as yet, not completely understood, but the low perinatal mortality rate in the normoglycemic pregnancies attests to the advantage of normal plasma glucose levels in pregnancy. The prognosis for the fetus of the pregnant diabetic is related to the degree of control of the maternal diabetes, and the results in this series support the principle that the goal for the management of the pregnant diabetic should be to keep her blood sugar level normal.[18] Karlsson and Kjellmer[17] have been able to show a dramatic reduction in perinatal mortality rates in diabetic pregnancies when the mean maternal blood glucose value was controlled at less than 100 mg. per 100 ml. (equivalent to 115 mg. per 100 ml. plasma glucose). Khojandi and colleagues[19] also concluded that perinatal mortality and morbidity rates were negligible when gestational diabetes was well controlled.

Evans and colleagues[20] reported a perinatal loss of 7 per cent when estriol excretion was normal and 23 per cent when low in their series of 93 diabetic pregnancies. In the present series (Table V) the combination of normoglycemia and normal estriol excretion (62.3 per cent of patients) selected a group of patients with a perinatal mortality rate of 0.4 per cent—the rate being 2.5 per cent when there was hyperglycemia ($p < 0.001$) and 1.6 per cent when there was hypoglycemia ($p < 0.05$). When estriol excretion was subnormal the perinatal mortality rate rose to 8.1 per cent when there was associated hyperglycemia, and was 9.5 per cent when hypoglycemia was present (Table V). Glucose tolerance testing and urinary estriol measurement were thus seen to be individually selective for pregnancies complicated by perinatal deaths, and by their combined use a high degree of selectivity was achieved, both for high and low degrees of risk.

Table IV. Incidences of small-for-dates babies according to glucose tolerance and urinary estriol excretion

Glucose tolerance test	*No. of patients*	*Incidence of small-for-dates babies according to urinary estriol excretion (per cent)*		
		Total series	*Normal estriol*	*Subnormal estriol*
Total series	2,000	9.8	7.9	27.7
Hyperglycemia	315	9.5	7.6	24.3
Normoglycemia	1,366	8.5	6.8	25.6
Hypoglycemia	346	15.6	12.5	38.1

Table V. Perinatal mortality rates according to glucose tolerance and urinary estriol excretion

Glucose tolerance test	*No. of patients*	*Perinatal mortality rate according to urinary estriol excretion (per cent)*		
		Total series	*Normal estriol*	*Subnormal estriol*
Total series	2,000	1.3	0.9	5.1
Hyperglycemia	315	3.2	2.5	8.1
Normoglycemia	1,366	0.6	0.4	2.5
Hypoglycemia	346	2.6	1.6	9.5

Hypoglycemia is a biochemical abnormality not previously described in pregnancy, and inasmuch as the present data have revealed associated pregnancy complications, detailed analysis of a larger series is to be undertaken. More data are required before principles of treatment of hypoglycemia in pregnancy can be enunciated. We believe that hypoglycemia will be proved to be a significant cause of previously unexplained intrauterine deaths. It is hoped that this hypothesis proves true, because very simple measures could provide effective therapy.

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Second-trimester spontaneous abortion, the IUD, and infection

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Nineteen cases of spontaneous second-trimester abortion associated with the intrauterine device (IUD) are reviewed and compared to 30 cases of spontaneous second-trimester abortion not associated with the IUD. Fifteen of the IUD's were Dalkon shields and four were Lippes loops. Sixteen of the 19 IUD-associated abortions began either with prolonged rupture of membranes or signs and symptoms of infection. Infection occurred in 18 or 19 of the cases (95 per cent) but only 30 per cent of the comparison group. Eleven major complications occurred in eight patients in the IUD group but only eight complications occurred among seven patients in the comparison group. It is suggested that when the IUD remains in utero during pregnancy, it may be a causal factor in second-trimester spontaneous abortion, and that intrauterine infection is the distinguishing characteristic of such cases. Mechanisms are suggested by which the IUD might cause infected abortion.

THE INTRAUTERINE device has come under recent suspicion as a cause of complications of pregnancy. In earlier reports, Scott[1] described ten deaths associated with the IUD. Two of these were definitely associated with pregnancy, although in both cases, the IUD had perforated the uterus. Lewit,[2] in a large cooperative study, showed high spontaneous abortion rates occurring in pregnancies with an IUD in utero. In a prospective study designed to show the influence on a pregnancy of removing an IUD, Alvior[3] showed that spontaneous abortion occurred in 48.44 per cent of cases when the IUD was left in situ but only 29.6 per cent of cases when the IUD (Lippes loop in this study) was removed early in pregnancy. Neither Lewit nor Alvior specified gestational ages of abortions occurring with an IUD in utero, nor were complications of these abortions described. Both studies concluded that an IUD should be removed early in pregnancy. Vessey and associates[4] have confirmed a high spontaneous abortion rate when IUD's remain in place.

More recently Shine and Thompson[5] followed 43 pregnancies occurring despite the presence of an IUD. Twenty-one (49 per cent) of these patients aborted spontaneously before 20 weeks. An additional four pregnancies or 9.3 per cent terminated with stillbirths. Febrile morbidity in the 21 spontaneous abortions was 33 per cent, far exceeding the hospital morbidity rate for all abortions of 4 per cent. Wiles and Zeiderman[6] followed eight patients with IUD's in utero. Seven patients had major maternal complications and six of these suffered fetal wastage. Dalkon shields outnumbered Lippes loops in this series seven to one. Hurt[7] confirmed the occurrence of septic pregnancy in five patients with Dalkon shields. Christian reported five deaths and seven other patients who were severely ill in the midtrimester with an IUD in place. All but two of these patients had shield-type devices. The report stresses "the insidious yet rapid manner in which these patients became ill," and points out the absence of early localized symptoms. Speculation is offered that the shield-type device predisposed toward early septicemia. Christian's[8] cases are gathered from different parts of the country and do not represent a series from a single locale. Information is not available concerning the prevalence of shield-type devices in the communities from which the patients came.

The A. H. Robins Company, [9] manufacturer of the Dalkon shield, has recognized the occurrence of

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Table I. IUD's found in the study

	No. of patients	*Per cent*
Dalkon shield	15	79
Lippes loop	4	21

midtrimester septic abortion and has withdrawn the device from the market. It recommends prompt removal of the device in the event of pregnancy, and consideration of therapeutic abortion if the IUD cannot be removed. The entire topic has been well reviewed in a Symposium following the recent Food and Drug Administration hearings on IUD's.[10]

In this study a series of 19 midtrimester spontaneous abortions are presented, all associated with IUD's. For comparison purposes, 30 cases of midtrimester abortion not associated with the IUD were studied. In comparing these two groups of patients, the evidence strongly suggests that IUD's cause a large proportion of the midtrimester spontaneous abortions with which they are associated, and that infection is the distinguishing characteristic of these abortions.

Materials and methods

All cases of midtrimester spontaneous abortions associated with an IUD, which occurred in Madison hospitals from 1971 through February, 1974, were identified by computer and manual search of discharge diagnoses and by asking physicians to identify patients. Nineteen were found. For comparison purposes, all cases of midtrimester spontaneous abortion not associated with an IUD occurring from 1971 through 1973 were similarly identified. Thirty cases were found.

The second trimester is defined here as being from the fourteenth to the twenty-seventh week, calculated from the last menstrual period. Febrile morbidity is defined as a temperature of 100.4° F. or greater recorded on two occasions six hours or more apart.

Results

Incidence. Forty-five midtrimester spontaneous abortions were identified during the three-year period, 1971 through 1973, including 15 of the 19 IUD patients. In that time, 11,548 births were recorded. Thus one midtrimester abortion occurred for every 257 births, or 3.9 cases per 1,000 births. By comparison, in 1961 the rate of midtrimester abortion was somewhat lower—one per 308 births.

Patient characteristics. Age, parity, percentage of nulliparous patients, and gestational age of pregnancy were similar in the IUD group and the comparison group. However, 12 in the comparison group had experienced previous spontaneous abortions (40 per cent) but only two of 19 IUD patients (11 per cent) had previously aborted spontaneously.

The IUD. Dalkon shields and Lippes loops were the only IUD's represented in the study (Table I). In no case was the IUD inserted after the onset of pregnancy. In no case was uterine perforation by the IUD suspected. The IUD's were all recovered with the products of conception or from the uterine cavity subsequent to the abortion. It is of interest that in three cases, x-rays of the pelvis prior to the abortion failed to reveal the presence of the IUD.

Presenting signs and symptoms. In eight of 19 IUD patients (42 per cent) rupture of the membranes, proved by conventional tests, preceded the abortion by more than 12 hours, and was the first sign of any complication in six of these pregnancies. Rupture of the membranes for days was common and the longest period of ruptured membranes was three weeks. In the comparison group only three cases or 10 per cent presented with ruptured membranes. When patients in the IUD group entered the hospital, 12 (63 per cent) had a tender uterus, 11 (58 per cent) complained of fever and chills, 10 (53 per cent) had purulent vaginal discharge, only nine (47 per cent) had audible fetal heart tones, and eight (42 per cent) reported or showed evidence of bleeding. In the comparison group, there was a higher incidence of bleeding (16 patients or 53 per cent) but other presenting complaints were significantly less frequent. The most common presenting complaints in the comparison group were abdominal cramping, bleeding, and absence of fetal heart tones (Table II).

Febrile morbidity. Febrile morbidity was present prior to the abortion in ten (53 per cent) of the IUD patients, but only six (20 per cent) in the comparison group. Five additional IUD patients had either undocumented fever or low-grade fever (100.0° to 100.4° F.) prior to the abortion. Several IUD patients had high temperatures exceeding 103° F. Following the abortion an additional three patients (15 per cent) became febrile, making the total febrile IUD patients 13 (68 per cent). Of the comparison group, only one became febrile after the abortion, making the total 23 per cent. Two of the febrile comparison patients had undergone cervical cerclage earlier in the pregnancy (Table II).

Laboratory data. White cell counts and differentials were obtained in eight IUD patients. Leukocytosis with a left shift (average WBC, 13,400) was the rule. Cultures were obtained in 18 of 19 IUD patients, taken from the endocervix, amniotic fluid, uterine cavity, or products of conception. *E. coli* was the most common

Table II. IUD patients and non-IUD patients compared

	IUD Group 19 patients	*Non-IUD (comparison) group 30 patients*
Presenting signs and symptoms:		
Proved rupture of membranes 12 hours prior to delivery	8 (42%)	3 (10%)
Bleeding	8 (42%)	16 (53%)
Purulent discharge	10 (53%)	5 (17%)
Fever and chills	11 (58%)	5 (17%)
Tender uterus	12 (63%)	13 (43%)
Audible fetal heart tones	9 (47%)	7 (23%)
Febrile morbidity:		
Prior to abortion	10 (53%)	6 (20%)
After abortion	5 (26%)	3 (10%)
Total of febrile patients	13 (68%)	7 (23%)
Complications:		
Excessive blood loss	4 (21%)	6 (20%)
Septic shock	2	0
Hemorrhagic shock	1	1
Pelvic cellulitis or thrombophlebitis	1	1
DIC	1	0
Late hemorrhage	1	0
Lung infiltrate	1	0
Total complications	11 complications 8 patients (42%)	8 complications 7 patients (23%)
Major final diagnosis:		
Abruption	4 (21%)	11 (37%)
Septic abortion	18 (95%)	9 (30%)
Fetal death	0	8 (27%)
Other	0	7 (23%)

organism isolated, but other dangerous pathogens were found as well. Blood cultures were obtained in eight patients, and four of these were positive. The organism was *E. coli* in every instance (Table III).

Mistaken diagnosis. On admission most of the patients were recognized to be experiencing a major obstetric complication. However, impending or septic abortion was not recognized in five IUD patients. Three were believed to have pyelonephritis, one patient was thought to have round ligament pain, and one patient was given an initial diagnosis of "cervicitis."

Treatment. Antibiotics were used in 18 of 19 IUD patients. Only nine of 30 (30 per cent) of the comparison group required antibiotics. Spontaneous labor occurred in about half of the IUD patients. Oxytocin stimulation prior to the abortion was required in ten IUD patients (53 per cent) and 13 comparison patients (43 per cent).

Operations were performed in ten IUD patients; D & C in nine patients (47 per cent) and hysterectomy in one patient with septic shock. In the comparison group, D & C was less often performed (seven patients—23 per cent but hysterectomy was necessary in three patients, and hysterotomy in two; all five major operations were done to halt severe hemorrhage.

Table III. Results of culture in 18 of 19 IUD patients

	No. of Patients
Uterine cavity endocervix, amniotic fluid:	
E. coli	8
S. fecalis	2
Alpha-strep	2
Beta-strep	1
Bacteriodes	2
S. aureus	1
Proteus	1
C. perfringens	1
Normal flora only	2
Blood:	
E. coli	4

Complications. No deaths occurred in this study. Of 19 IUD patients, four experienced excessive blood loss (estimated greater than 1,000 c.c.) and one of these suffered hemorrhagic shock. Two patients had septic shock, and one of these also had disseminated intravascular coagulation and a prolonged course of pelvic cellulitis. Late hemorrhage and lung infiltrate occurred in one patient each. In all, eight patients or 42 per cent experienced 11 major complications. In the compari-

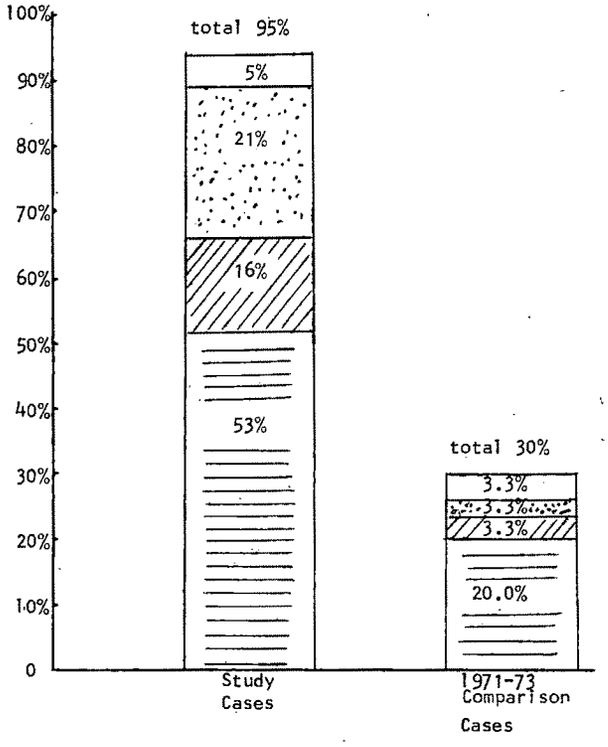


Fig. 1. The evidence for infection in 19 IUD patients and 30 non-IUD (comparison) patients who aborted spontaneously in the mid trimester. Clear background, pathologic evidence of infection only; stippled background, clinical evidence of infection only, i.e., purulent discharge, undocumented fever and chills, foul-smelling placenta, etc.; diagonal stripes, documented fever > 100.4° F. following abortion; horizontal stripes, documented fever > 100.4° F. before abortion.

son group, six patients had excessive blood loss, and one of these went into shock. Five of these patients underwent hysterectomy or hysterotomy. One patient had a prolonged course of pelvic cellulitis and pelvic thrombophlebitis. In all, seven of 30 patients in the comparison group (23 per cent) experienced major maternal complications. Fetal complications included two live immature fetuses in the IUD group and two severely deformed fetuses in the comparison group. No fetus in either group survived. Three patients in the comparison group aborted following placement of a cervical cerclage for incompetent cervix (Table II).

Major final diagnoses. All but one of the 19 IUD patients were discharged with a diagnosis indicating septic abortion. Three of these had a diagnosis of abruptio placentae as well. One patient had a diagnosis of abruptio placentae uncomplicated by infection. Of the 30 comparison patients, abruptio placentae accounted for 11 (37 per cent) and septic abortion nine (30 per cent). Intrauterine fetal death for various reasons accounted for eight patients (27 per cent) and the remainder of the diagnoses included incompetent cervix (2 patients), incompetent cervix with failure of cervical cerclage (3 patients), bicornuate uterus (1 patient), and premature labor of unknown etiology (1 patient) (Table II).

Comment

Does the IUD cause abortion? Direct evidence is not available from this study. Two related pieces of evidence, however, strongly support causality; first, that the onset of the abortion is different in the two groups of patients and, second, that a single unifying factor is common to all or most of the IUD patients.

Ten of the 19 IUD patients presented with signs and symptoms of infection as the first evidence that the pregnancy was complicated. Prolonged rupture of membranes, well known to predispose to infection, was proved in another six patients as the first sign of impending abortion.

Two patients aborted before they reached the hospital. Their presenting signs and symptoms are unknown. Only one patient presented with bleeding. In the comparison group, signs and symptoms of infection and prolonged rupture of membranes were uncommon modes of presentation.

The single unifying factor for the IUD patients is clearly infection. Febrile morbidity, the best single indicator of infection, was present in 13 of 19 patients or 68 per cent. Four patients, or an additional 21 per cent, had clinical evidence of infection such as purulent discharge, foul-smelling products of conception, or history of fever and chills without a documented temperature elevation. One additional patient had evidence of infection only on pathologic examination of the products of conception, that is, the placental tissue was shown to have acute chorioamnionitis in the absence of fever or clinical signs of infection. In all, 18 of 19 IUD patients or 95 per cent showed evidence of infection.

On the other hand, among the comparison group seven patients (23 per cent) were febrile. Of these, two patients had undergone cervical cerclage earlier in their pregnancy. One additional patient exhibited signs and symptoms of infection without become febrile. One more patient carried a diagnosis of chorioamnionitis on the basis of pathologic examination only. In all, nine of the 30 patients (30 per cent) showed evidence of infection and two of these had undergone cervical surgery (Fig. 1).

Thus, infection as a concomitant part of the abortion process is almost universally associated with the IUD. It is not, as the comparison group shows, a routine secondary complication of midtrimester abor-

tion. Since evidence of infection or ruptured membranes is present at the onset of abortion in the IUD patients, the IUD is strongly implicated as the cause of infected abortion.

It is possible that the difficulties of making an early diagnosis and the fulminating course of septic abortion in the IUD patients have been overemphasized. The diagnosis was straightforward in most of the IUD patients in this series. In five patients the initial diagnosis was incorrect, but was reversed in every case except one, soon enough to prevent permanent damage. Certainly, septic abortion is potentially a grave complication, yet only two patients in this series could be described as severely ill. One underwent hysterectomy for septic shock 24 hours after she was admitted with a mistaken diagnosis of pyelonephritis and a second patient had septic shock and disseminated intravascular coagulation. She was promptly treated with antibiotics and dilatation and curettage, but nevertheless developed a pelvic cellulitis requiring over a month of hospitalization and antibiotics. Most of the IUD patients were not severely ill and responded promptly to antibiotics and aggressive efforts to empty the uterus.

Whether a specific IUD is especially associated with infected abortion is an open question. The Dalkon shield has been under particular suspicion and the evidence implicating it seems to be increasing. In this series, although Dalkon shields outnumber Lippes loops 15 to four, the proportion of different IUD's in the general population is not known. Dalkon shields have been widely used in the Madison area.

The mechanisms whereby IUD's might cause septic abortion are a matter for speculation. With the Dalkon shield, the prongs are suspected to cause localized changes predisposing to infection. The relative rigidity of the Dalkon shield may cause shearing forces between the endometrium and the chorioamnion. Either the IUD tail may act as a nidus of infection as it is drawn up out of the vagina into the uterus by the enlarging pregnancy or it may act as a wick, drawing up contaminated vaginal contents. That this occurs with the Dalkon shield in vitro with dye and bacterial suspensions has been elegantly demonstrated by Misheli and associates.[10].

This study is believed to represent the total experience of this community with IUD-associated midtrimester abortions. If the Madison experience has validity in other communities, midtrimester-infected abortion associated IUD's is far more common than previously suspected. It is a danger which should be considered, and of which the patient should be warned, at every IUD insertion. The threat of infected abortion argues for the removal of an IUD in early pregnancy in every case. If simple removal is not possible, consideration should be given to therapeutic abortion. Should intrauterine infection in the presence of an IUD occur, a high index of suspicion will lead to proper diagnosis and effective treatment.

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Quantitation of uterine activity in 100 primiparous patients

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An on-line technique for quantitating uterine activity by measuring the total area under the intrauterine pressure curve was utilized during the labor of 100 primiparous patients. Uterine activity was measured in uterine activity units (one UAU = one Torr minute). UAU's were calculated for each patient in both cumulative and noncumulative fashions. The rate of uterine activity (UAU per unit of time) increased as labor progressed, yet the total UAU's expended at each centimeter of dilatation progressively decreased with advancing dilatation. Thus, the rate of cervical dilatation increased faster than could be accounted for by the increase in uterine activity. The implication being that the efficiency of uterine contractions increases with advancing labor. It no longer appears reasonable to refer to labor in terms of elapsed time. Information regarding the "pattern of cervical dilatation" and uterine activity appears to be much more meaningful to the evaluation of progress in labor. Quantitation of uterine activity may provide useful information for the ongoing management of labor. Quantitation of UA does provide a means of evaluating the effects of specific events such as medication, anesthesia, and manipulation on UA.

THE FORCES OF labor have been the subject of interest and clinical investigation for decades. Methods of evaluating uterine activity (UA) have progressed from manual palpation (of uterine contractions), to tocodynamometry, to the direct measurement of intrauterine pressure (IUP).

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It seems reasonable to accept the premise that UA is related to progress in labor. Until recent years, the difficulty in accurately measuring UA precluded it as a clinical method for evaluating progress in labor. The most popular method of assessing adequacy of UA is the evaluation of progressive cervical dilatation. The graphic portrayal of cervical dilatation plotted against time was introduced by Friedman[1] in 1954. Utilizing this technique, an expected pattern of cervical dilatation during normal labor has been established which provides a means of recognizing abnormal progress. This serves to alert the physician to a potential problem but in no way identifies the underlying etiology. It has been claimed that slow progress in cervical dilatation is commonly associated with less than optimal UA.[2] Conversely, the cervical dilatation pattern may be abnormal despite apparently adequate UA in patients with fetal malpresentation or fetopelvic disproportion.[3] In order to intelligently interpret cervical dilatation patterns, one must be able to quantitate, in some manner, the strength of the uterine contractions.

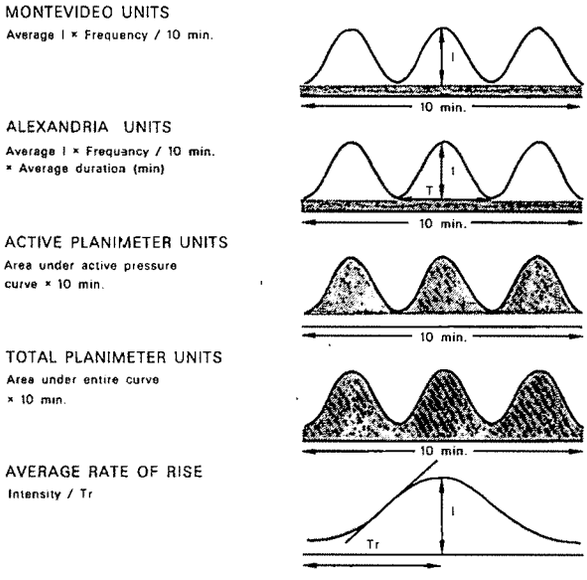


Fig. 1. Methods for quantitation of uterine activity.

Various methods have been utilized in an attempt to quantitate UA from intrauterine pressure recordings as shown in Fig. 1. Manual methods of calculation, such as the Montevideo and Alexandria units, are based on the measurement of the amplitude of the uterine contractions above base-line tone multiplied by the number of uterine contractions per ten minutes. (The Alexandria Unit also includes the average duration of the contractions in minutes.) The manual method has the disadvantage of being very time consuming. Planimeters can very accurately measure the area under the uterine pressure curve, but the method is also slow and time consuming. Analogue and digital computers can provide accurate measurements of UA by integrating the area under the entire uterine pressure curve or under only the "active area"; however, the equipment is expensive and cumbersome.

For the past three years, a simple on-lime technique for measuring the total area under the uterine pressure curve has been utilized in Women's Hospital, Los Angeles County—University of Southern California Medical Center.[4] This system integrates the total area (including uterine tone and contractions) rather than any fraction or segment of the intrauterine pressure. The entire area under the IUP curve was utilized since it seems reasonable that both tone and contractions may affect the rate of cervical dilatation. Additionally, it is technically much simpler to quantitate the total area than to evaluate the uterine contractions separately from the base-line tone. By quantitating UA in this manner, no comment can be made regarding the base-line uterine tone or the frequency and amplitude of uterine contractions. The equipment utilized is relatively inexpensive and easy to operate, and the data are quantitative and immediately available.

This paper presents our experience utilizing this on-line method for quantitating UA in 100 primiparous labors.

Table I. Patient population

Obstetric condition	*No. of Patients*
Normal	49
Toxemia	24
Prolonged pregnancy	8
Premature	6
Meconium	5
Diabetes	3
Maternal heart disease	2
Miscellaneous	3
Total	100

Table II. Cervical dilatation at onset of monitoring

Dilatation (cm.)	*No. of Patients*
3	8
4	20
5	47
6	12
7	7
8	6
Total	100

Material and methods

One hundred primiparous patients with the fetuses in the vertex presentation, whose labors were monitored in the Perinatal Intensive Care Delivery Unit, were selected for evaluation. Patients selected for fetal monitoring during labor in this unit are generally high-risk obstetric patients who have a higher incidence of obstetric complications than the normal patient population as shown in Table I. All patients studied had IUP measured with the use of a transcervical open-end fluid-filled catheter, connected to a Statham pressure transducer, positioned at the level of the anterior chest wall. The area under the uterine pressure curve was integrated with the use of a voltage-controlled oscillator (VCO). A digital print out of the uterine activity (measured in millimeters of mercury per minute, or Torr minutes) was obtained every ten minutes. A uterine activity unit (UAU) equal to one Torr minute will be used to express uterine activity in this study.[5]

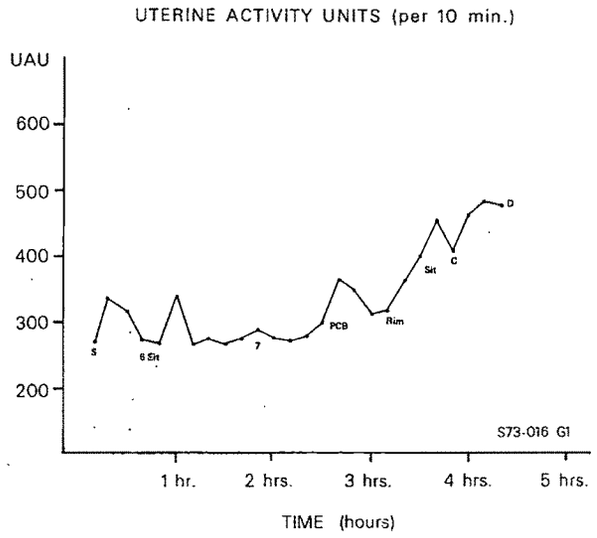


Fig. 2. The noncumulative uterine activity units per ten minutes from 5 cm. to delivery (one patient). The associated effect of events such as vaginal examination (5, 6, Rim, etc.), paracervical block *(PCB)*, and position change *(Sit)* on uterine activity are clearly shown.

Table III. Number of examinations at each centimeter of cervical dilatation in which there was an increase or decrease in mean uterine activity compared with previous dilatation

	Dilatation (cm.)							
No. changes	*3-4 to 4-5*	*4-5 to 5-6*	*5-6 to 6-7*	*6-7 to 7-8*	*7-8 to 8-9*	*8-9 to 9-C*	*9-C to C-D*	*Total*
Increases	3	11	28	28	31	35	70	206
Decreases	1	4	5	19	15	10	8	62
Total	4	15	33	47	46	45	78	268

No attempt was made to alter the usual management of labor and delivery as performed in the Perinatal Intensive Care Delivery Unit. Individual variations in patient care were limited by protocols which provide strict guidelines for labor management in this unit. Patients received intravenous analgesia (Demerol and occasionally Demerol plus Phenergan) as clinically indicated. Paracervical block and/epidural anesthesia were used in selected cases. Magnesium sulfate dosage, when indicated, was given via continuous infusion pump. A policy of active labor management was practiced. If the cervical dilatation was not progressing and UA on clinical grounds was considered to be "inadequate," a low-dose intravenous oxytocin infusion was promptly instituted by use of an infusion pump. One half of all the patients received oxytocin at some time during labor; ten per cent of these for induction and 90 per cent for augmentation. Vaginal examinations were performed by one examiner in each patient for consistent assessment of cervical dilatation. All patients reached full dilatation. Two patients were delivered by cesarean section after two hours of good labor in the second stage with failure of descent of the presenting part.

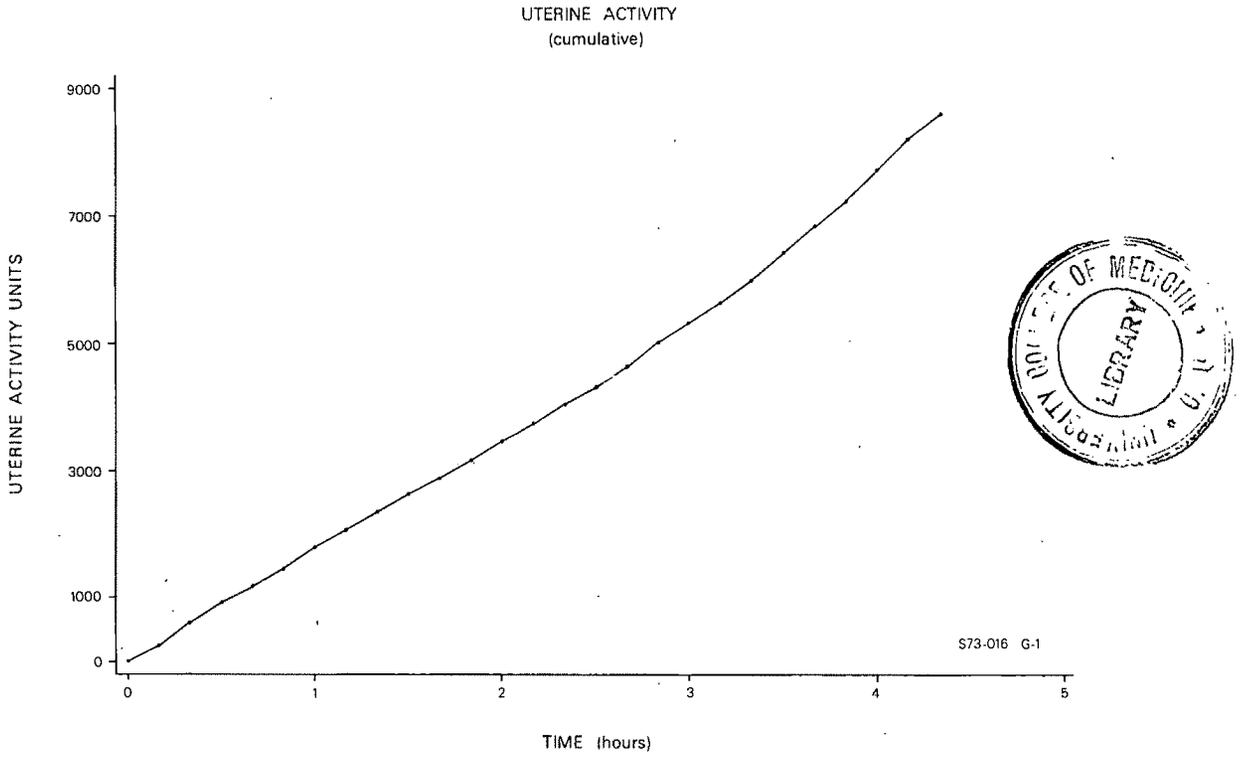
Results

The cervical dilatation at the onset of monitoring ranged from 3 to 8 cm. (Table II). The longest monitored labor was eleven hours and fifty-six minutes, and the shortest was 56 minutes.

The uterine activity (reported in UAU's) was calculated for each patient in both cumulative and noncumulative fashions. The UAU's from the "normal" labor of a 16-year-old patient are shown in Fig. 2, plotted in a noncumulative fashion (UAU per ten minutes) from 5 cm. dilatation until delivery. UAU's progressively increased as labor progressed, although there were notable variations in the UAU's when successive ten-minute intervals were compared. The apparent effects associated with vaginal examination, medications, and position change on uterine activity are easily seen. The uterine activity from the same patient plotted in a cumulative manner (Fig. 3) forms a slope that is essentially linear with an increase in the slope near the end of the first stage and during the second stage of labor.

The variation of cervical dilatation at the onset of UA monitoring and in the length of labor make it difficult to compare UA at corresponding elapsed times in different patients. Delivery does provide a common end point and one may retrospectively plot UAU's for each ten minute interval for a relative comparison. A mean curve for the noncumulative UA for the six hours prior to delivery was plotted for the 100 labors (Fig. 4). There was a progressive increase in UAU's as labor progressed. The mean UAU's increased from 210 per ten minutes six hours prior to delivery to 375 per ten minutes at delivery.

Possibly a more useful way to evaluate UA is to calculate the UAU's required for each centimeter of cervical dilatation. The mean UAU's per ten minutes required to dilate the cervix from 3 to 4 cm., 4 to 5 cm., etc., were calculated. During these 100 labors, 428 vaginal examinations were performed in which there was a change in cervical dilatation. The dilatation had advanced 1 cm. since the previous examination in 318, 2 cm. in 76, and 3 cm. or more in 34. The UA data for changes of 3 cm. or more between examination was



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Fig. 3. Cumulative uterine activity units recorded every ten minutes from 5 cm. dilatation until delivery (one patient).

excluded because no accurate estimation could be made of the amount of UA expended at each centimeter of dilatation. When there was a 2 cm. change between examinations, the total UAU's were calculated and divided equally between the two centimeters. The mean UAU's per ten minutes at each centimeter of dilatation for all the patients in the study are shown in Fig. 5. The mean UAU's per ten minutes for patients grouped according to the cervical dilatation at the onset of monitoring showed progressive increases in each group as labor advanced. There were 268 examinations in which cervical dilatation advanced 1 cm. on two successive examinations. With this information a comparison of the mean UAU's per ten minutes at each centimeter can be made. Table III shows the number of examinations and the increases and decreases in mean UAU's with successive dilatation. The number of increases greatly outnumbers the decreases at each centimeter of cervical dilatation. Over all, the mean UAU's per ten minutes decreased in 23 per cent of examinations as labor progressed. However, in only five cases did the mean UAU's per ten minutes decrease twice in a row with progressive cervical dilatation. There were no patients with a decrease in the mean UAU's if cervical dilatation progressed more than 1 cm. between examinations (indicating rapid laxor progression). Although the rate of UA (UAU's per ten minutes) increased as labor progressed, the mean total UAU's required to effect cervical dilatation decreased with each successive centimeter of dilatation as shown in Fig. 6. The mean total UAU's were calculated by adding the mean UAU required to dilate each centimeter from 3 cm to delivery. Although there were only eight patients in the 3 to 4 cm. group, the 4 to 5 cm. group included these patients plus the 20 patients in whom monitoring was started at this dilatation, etc. A mean total of 11,536 UAU's was required to progress from 3 cm. to delivery in this group of patients. The UAU's necessary to accomplish delivery after complete dilatation were approximately one-fifth the total UAU's for the monitored labor.

The rate of cervical dilatation in centimeters per hour (slope of cervical dilatation) plotted at each centimeter of cervical dilatation for all 100 patients is shown in Fig. 7. It is obvious that as labor progresses, the slope of cervical dilatation progressively increases. The mean slope of cervical dilatation was then plotted against the mean UAU's per ten minutes at each centimeter of dilatation (Fig. 8). For this evaluation, the

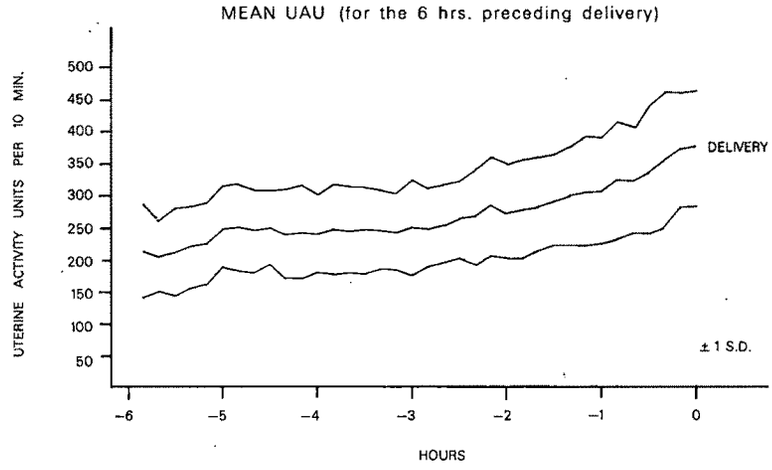


Fig. 4. Mean UAU's for 100 patients plotted retrospectively every ten minutes for six hours preceding delivery.

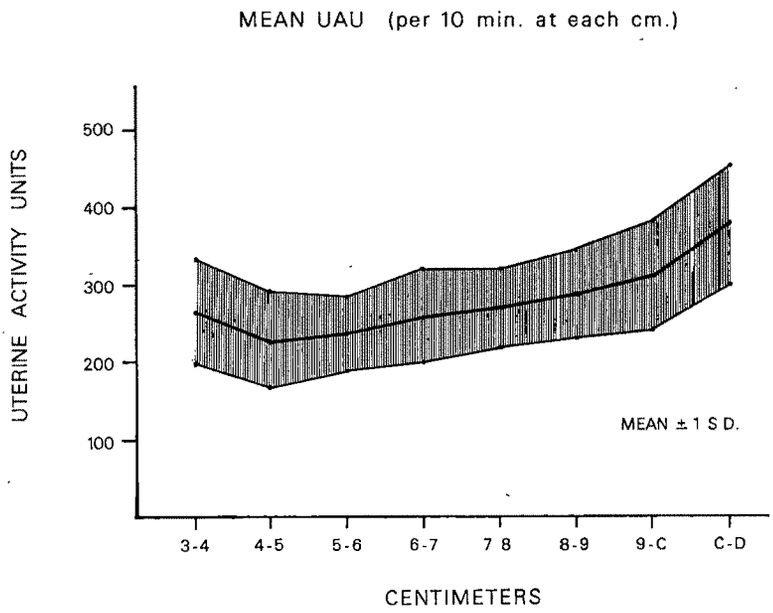


Fig. 5. Mean UAU's per ten minutes at each centimeter of dilatation for 100 patients.

data for changes of 3 cm. or more between examination were excluded for the reasons described earlier in the paper. With the exception of the 3 to 4 cm. group in which there were only eight samples, there was a direct correlation between the mean UAU's at each centimeter of dilatation and the slope of cervical dilatation.

When comparing UAU's in patients receiving oxytocin and those not receiving oxytocin, lower mean UAU's per ten minutes were found in patients receiving oxytocin at each centimeter of cervical dilatation. As labor progressed, these differences diminished, and at complete dilatation the mean UAU's were the same in the two groups (Fig. 9).

Comment

There are a number of factors that influence labor and any attempt to predict the course of labor with the

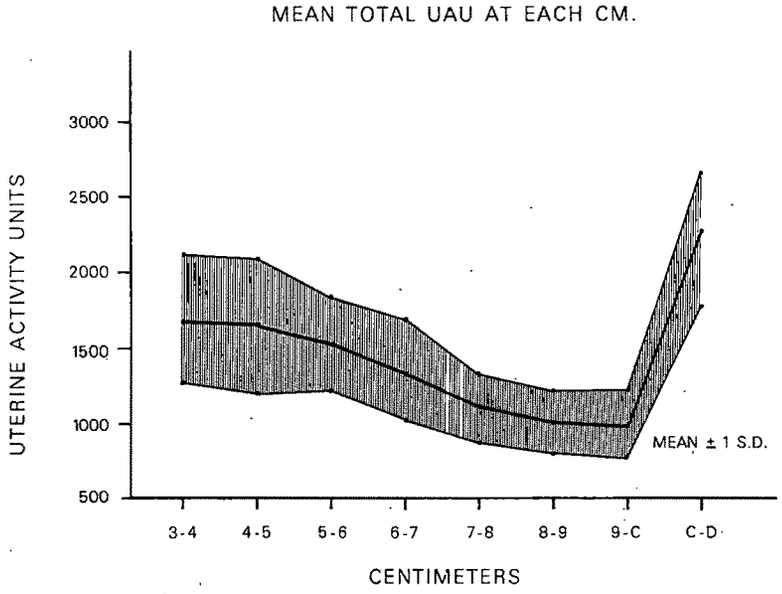


Fig. 6. Mean total UAU's expended at each centimeter of dilatation in 100 labors.

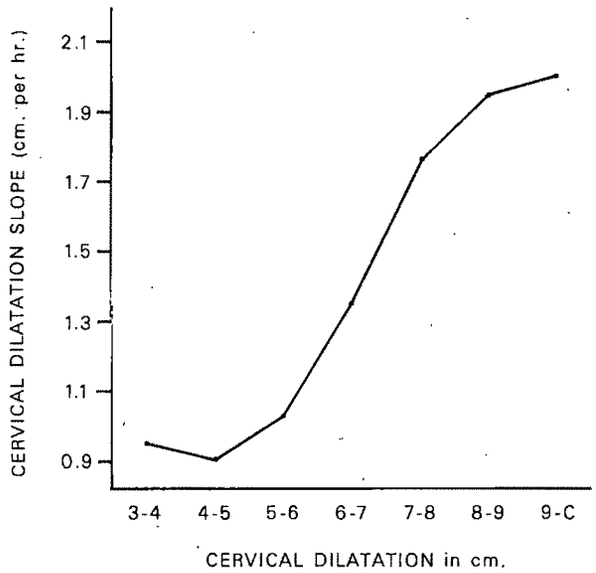


Fig. 7. The slope of cervical dilatation at each centimeter of dilatation (100 patients).

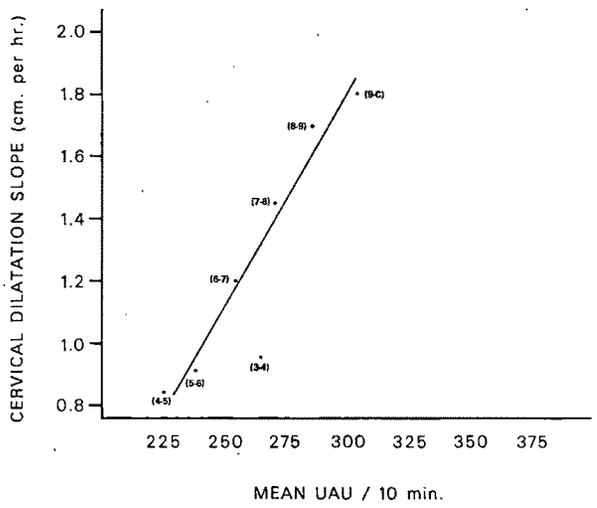


Fig. 8. The slope of cervical dilatation versus mean UAU's per ten minutes at each centimeter of dilatation (100 patients). (The numbers in parentheses indicate the centimeters of cervical dilatation.)

use of only one parameter is hazardous. However, in the absence of disproportion, progress in labor (cervical dilatation) will likely be proportional to the amount and quality of uterine contractions. Guidelines have previously been established for the expected rate of cervical dilatation per unit of time (hours) during normal labor. The amount of uterine activity required for normal labor has not been established. Until recently, instrumentation which could provide this information was not readily available for clinical use in the ongoing management of labor. Quantitation of UA from the IUP recording can provide this information.

Of the 11,536 UAU's required for labor from 3 cm. to delivery, approximately 20 per cent were required for the second stage of labor. Nine thousand three hundred and thirty-one UAU's were required to advance from 3 cm. to complete dilatation. Fifty-three per cent of this total was required from 3 to 6 cm. and

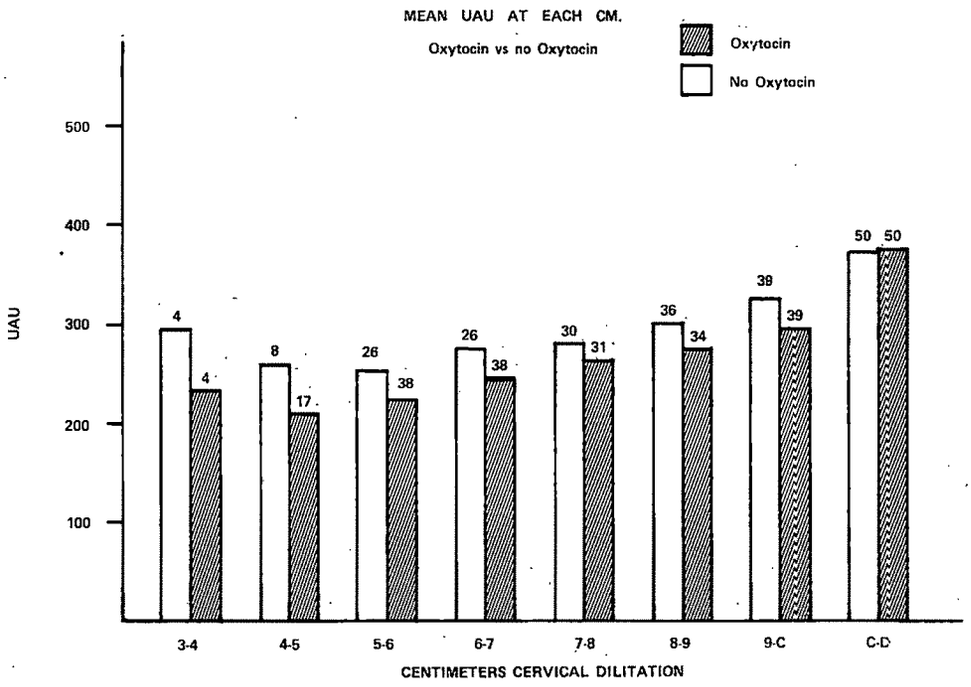


Fig. 9. The mean UAU's at each centimeter of dilatation comparing patients with and without oxytocin.

47 per cent from 6 cm. to complete dilatation. This is in contrast to the data of Burnhill[6] who found that 80 per cent of the "work" of the first stage was expended to progress from 3 to 6 cm. While the rate of uterine activity (UAU per unit of time) increased as labor progressed, the total UAU's expended at each centimeter of dilatation progressively decreased with advancing dilatation. Thus, the rate of cervical dilatation increased faster than could be accounted for by the increase in UA. It appears that much of the UA during the first stage relates to effacement and, once the cervix reaches a certain dilatation, there is rapid progression. This apparently coincides with the phase of maximum slope of cervical dilatation as described by Friedman.[7] The implication is that the efficiency of uterine contractions increases with advancing labor. There is a direct correlation between the slope of cervical dilatation and UAU's.

It no longer appears reasonable to refer to labor in terms of elapsed time. Information regarding the "pattern of cervical dilatation" is much more meaningful to the evaluation of progress in labor. Quantitation of UA might provide useful information to help in the ongoing management of labor. During the course of labor, the mean UAU's at any cervical dilatation can be calculated. The rate of UA (mean UAU's per ten minutes) can be compared to any centimeter of dilatation against the rate of UA for a preceding dilatation. If labor is not progressing adequately and the rate of UA is not increasing, appropriate therapy can be initiated to improve UA. If the mean UAU's are increasing, attention can be directed toward other possible causes of inadequate progress in labor.

Quantitation of UA provides a means of evaluating the effects of specific events such as medication, anesthesia, and manipulation on UA. This allows for an objective comparison of UA between individual patients and groups of patients. Quantitation also permits the use of a systems approach in the evaluation of labor.

Although the patients evaluated in this study do not represent "ideal" or "pure" study groups, the strength of the correlation between UAU's and the cervical dilatation pattern suggests that this might be a useful adjunct for assessing labor. Additional observations are necessary for assessing the correlations between UA and specific cervical dilatation patterns.

A method is now available which provides quantitation of UA for utilization in the evaluation of labor. The information is accurate and immediately available. Quantitation of UA provides objective data on which to base decisions for the ongoing management of labor.

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VIII World Congress of Gynecology and Obstetrics

The VIII World Congress of Gynecology and Obstetrics will be held in Mexico City, Mexico, October 17-22, 1976.

Payment of the enrollment fee entitles both members of the FIGO and nonmembers to attend the scientific sessions and join in the social activities and to receive a copy of the Congress program and the book of abstracts.

Only active members of national federations, societies, or colleges affiliated with the FIGO may present free communications, scientific exhibits, and films or take part in scheduled discussions.

Registered accompanying persons are welcome at all social events as well as the tours especially organized for them.

For enrollment, form 1 must be completed and sent in with payment to the General Secretariat before July 31, 1976.

All payments must be made through bank draft to the order of "VIII Congreso Mundial de Ginecología y Obstetricia." The General Secretariat will mail back a receipt, exchangeable for the documents at the Congress Building as of Friday, October 15, 1976, from 9:00 A.M. to 5:00 P.M. Payment should be sent to General Secretariat, Avenida Baja California 317, México 11, D. F., Mexico.

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Immunoglobulins and beta-1-C globulin in hydatidiform mole vesicles

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Qualitative and quantitative determinations of immunoglobulins (Ig's) and beta-1-C globulin were carried out in fluid vesicles of hydatidiform moles and carrier mothers. The mean concentration of IgG was 9.9 per cent, IgA had 14.3 per cent, in relation to maternal values. No IgM concentrations were detected in molar fluid. Beta-1-C globulin showed 60.5 per cent concentration in relation to mother's serum. It is suggested that in trophoblast of molar vesicles a selective transudate migration of Ig's by a mechanism of pinocytosis takes place to the vesicular fluid.

THE TROPHOBLAST is the first tissue to become differentiated in mammals.[1] Its functions include: hormone synthesis. nidation of the blastocyst in the endometrium, the early metabolic exchanges between mother and embryo. Immunologic isolation of the embryo has also been attributed to the trophoblast, accounting for the acceptance of the former as an allograft in the uterus.[2–4] This isolation, however, does not prevent maternal humoral antibodies from crossing the trophoblast in both directions. Immunoglobulin synthesis, on the other hand, occurs in the fetus at an early gestational age and some fetal immunoglobulins also pass through the trophoblast to the maternal circulation.[5] This dual exchange of immunoglobulins renders the evaluation of maternal immunoglobulins entering the embryonic circulation difficult. The fluid of vesicles of hydatidiform moles was chosen in order to study trophoblastic permeability to immunoglobulins because there is no embryo or embronic circulation in these pathologic pregnancies. Previous studies related to electrolytes and plasma proteins have been performed by McKay and associates,[6, 7] using this model. We hereby report the concentration of immunoglobulins in hydatidiform mole fluid vesicles. Values were compared with the same immunoglobulins in maternal sera.

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Reprint requests: Dr. Héctor Márquez-Monter, Apartado Postal 73-032, Mexico 73, D.F., Mexico.

Material and methods

Eleven recently delivered hydatidiform moles were obtained from the Hospital de Gineco-Obstetricia Number 2 of the C.M.N., I.M.S.S. Careful examination did not disclose embryos in these specimens. Grossly the moles were constituted by multiple vesicles with diameters ranging from 0.3 to 4 cm.; the largest vesicles were washed minimally in physiologic saline and were punctured with a needle for the extraction of fluid. Serum was also obtained from the patient at the moment of delivery. Unconcentrated vesicular fluid and the corresponding maternal serum were analyzed by microimmunoelectrophoresis,[3] using rabbit anti–whole-human serum and anti-human immunoglobulins (IgG, IgA, IgM) antisera. Quantitative determination of immunoglobulins and beta-C globulin in vesicular fluid and in serum was done by single radial immunodiffusion in agar plates.[9]

Results

Immunoglobulins G, A, and M, and beta-1-C globulin concentrations in vesicular fluid and sera are shown

Table I. Immunoelectrophoresis in hydatidiform moles

Case	*No.*	*IgG*		*IgA*		*IgM*	*Beta-1-C*	
1	S*	2,000		600		230	280	
	M	460	23%	94	15.6%	0	35	12.5%
2	S	1,450		150		60	125	
	M	62	4.27	22.5	15.1	0	19	15.1
3	S	890		130		150	180	
	M	48	5.4	21	16.1	0	19	10.6
4	S	850		320		100	150	
	M	60	7.04	0	0	0	27	18.1
5	S	1,100		105		100	115	
	M	180	16.3	20	19	0	21	18.2
6	S	1,300		480		150	110	
	M	24	1.8	23	4.78	0	18	16.4
7	S	1,000		210		180	150	
	M	115	11.5	37	17.9	0	25	16.6
8	S	550		260		170	105	
	M	12	2.2	26	10	0	19	18.2
9	S	700		100		52	140	
	M	180	25.6	41	41.6	0	70	22.4
10	S	550		240		34.5	115	
	M	38	6.9	18.5	7.7	0	16.5	14.5
11	S	760		150		68	140	
	M	44	5.8	14.5	9.3	0	16.5	11.8
Average	S	1,013.64		249.55		117.68	155.45	
	M	111.18		28.86		0	26	
+	S	434.12		161.25		62.43	56.90	
− S.D.	M	129.42		24.14		0	15.61	

*S = maternal serum; M = molar fluid.

in Table I, as well as the percentage of vesicular Ig's when compared to the maternal serum values. The mean concentration of IgG in molar fluid was 9.9 per cent of the maternal serum value (with a range of 1.8 to 25.6 per cent). For IgA, the mean concentration was 14.3 per cent of the maternal serum value with a range of 0 to 41.6 per cent. No IgM was detected in the 11 hydatidiform moles included in this study, whereas the concentration of IgM in the corresponding maternal serum was normal, except in one case where a low value of 34.5 mg. per 100 ml. was found. Finally, mean concentration of beta-1-C globulin was 60.5 per cent of the maternal value with a range of 10.6 to 22.4 per cent.

Immunoelectrophoretic analysis revealed a normal pattern for all the maternal sera except the case that showed low IgM (Fig. 1). All molar fluids revealed the presence of albumin, alpha-1, alpha-2, and beta-globulins, beta-1-C globulin, IgA, and a low concentration band of IgG in a slightly cationic position. No IgM precipitation band was found in the immunoelectrophoresis analysis of vesicular fluid. Those cases with concentration of IgG lower than 100 mg. per 100 ml. disclosed discrete duplication of the IgG band, probably a result of a progressive precipitation.[10]

Comment

Fetal serum of a gestational age similar to the hydatidiform moles included in this study (23 to 30 weeks), usually contains IgG of maternal origin amounting to 4 to 8 percent of the concentration in maternal serum.[11] Normally no IgA or IgM is found in fetal serum, although both can be found in cases of intrauterine infection. Fetal serum of this age contains approximately 15 mg. per 100 ml. of beta-1-C globulin, all of which is of fetal origin.

These data suggest—albeit no definite proof is offered—that immunoglobulins and beta-1-C globulin in hydatidiform mole fluids result from a transudation of maternal serum through a mechanism possibly including pinocytosis. First, because it contains IgA in considerable amount (19.3 per cent from the maternal values), and this immunoglobulin neither is synthesized by the fetus in normal conditions nor crosses the placenta in measurable amounts. If fetal infection is claimed to explain the presence of IgA in hydatidiform

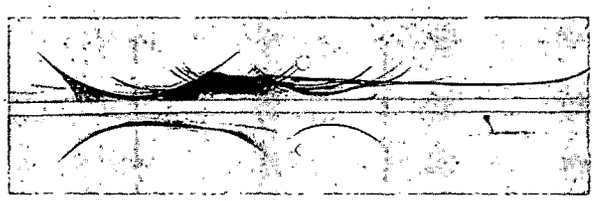


Fig. 1. Immunoelectrophoresis of molar fluid (lower) and maternal serum (upper) with whole antiserum.

moles, one has to consider that the same condition would also lead to the presence of IgM, and none of the cases revealed this immunoglobulin in the vesicular fluid. The high molecular weight of IgM, however, could explain its paucity or absence in a transudate fluid. On the other hand the presence of IgA could be due to a leakage of this immunoglobulin through an abnormal trophoblastic barrier of the hydatidiform mole.

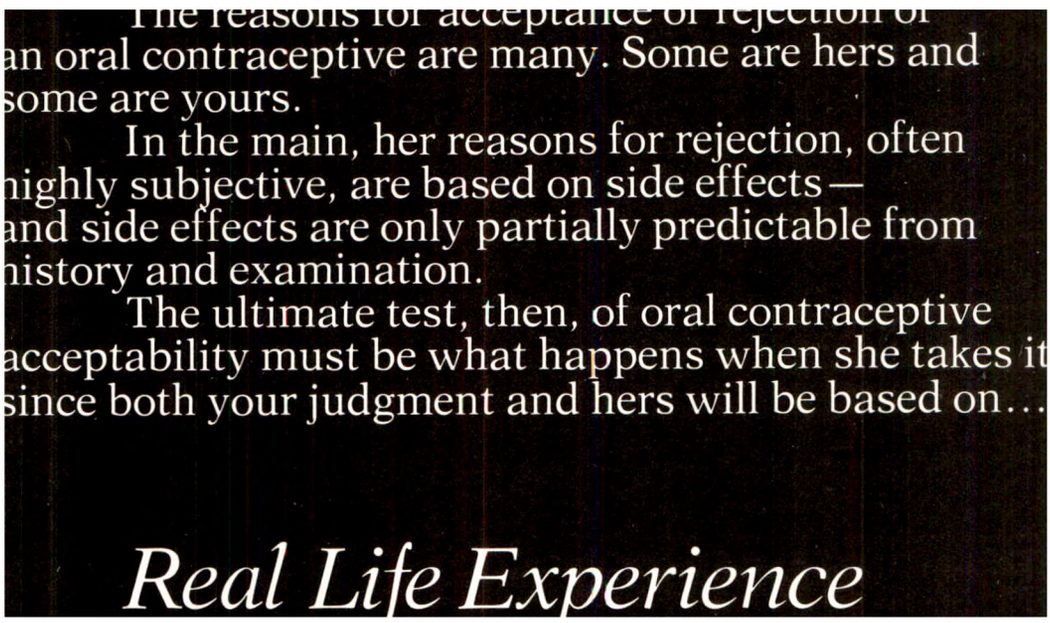
Beta-1-C globulin is synthesized by the fetal liver from the twelfth week of gestation onwards, and its concentration in fetal serum in the second trimester is

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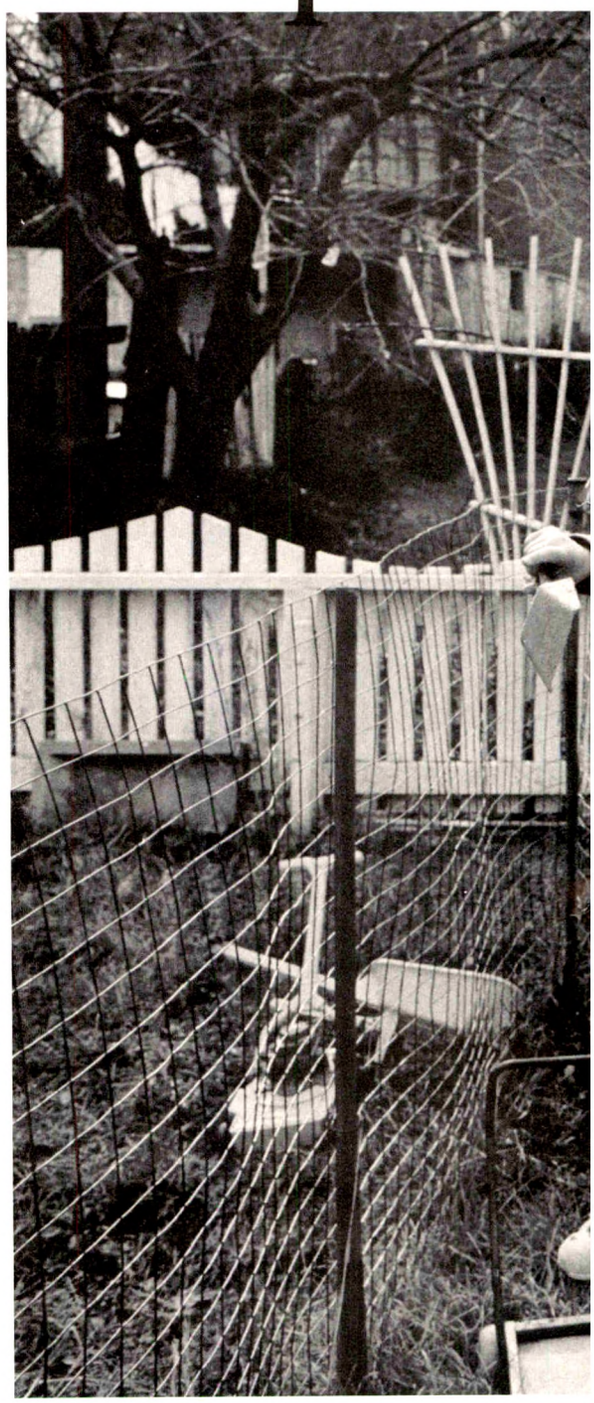
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The reasons for acceptance or rejection of
an oral contraceptive are many. Some are hers and
some are yours.
In the main, her reasons for rejection, often
highly subjective, are based on side effects—
and side effects are only partially predictable from
history and examination.
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acceptability must be what happens when she takes it
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ZORANE 1/50 is a highly effective and generally well-tolerated oral contraceptive—providing the estrogen content and progestin:estrogen ratio that appears to be highly acceptable to the majority of women.

From clinical studies: In a study population of 1156 women, 25,983 cycles, the incidence of nausea and vomiting was 3.3%. Actual patient dropout rate* due to nausea and vomiting was 2.9% (33 subjects.) Overall patient dropout rate was 14.4% due to all adverse reactions. Serious reactions, as well as minor adverse reactions, have been reported following the use of all oral contraceptives. Before prescribing, the complete product information should be consulted for *special note, contraindications, warnings, precautions* and *adverse reactions*. Thereafter, the physician should remain alert to the earliest manifestations of any serious disorder. Should any occur or be suspected, the oral contraceptive should be immediately discontinued.

The woman profiled here is an actual ZORANE 1/50 user. However, not all women can be expected to have the same tolerance for side effects, should any occur. (Data on file, Lederle Laboratories)

*Dropout rates are based on total patient population (1,156); incidence reflects only those patients who completed one or more cycles (956).

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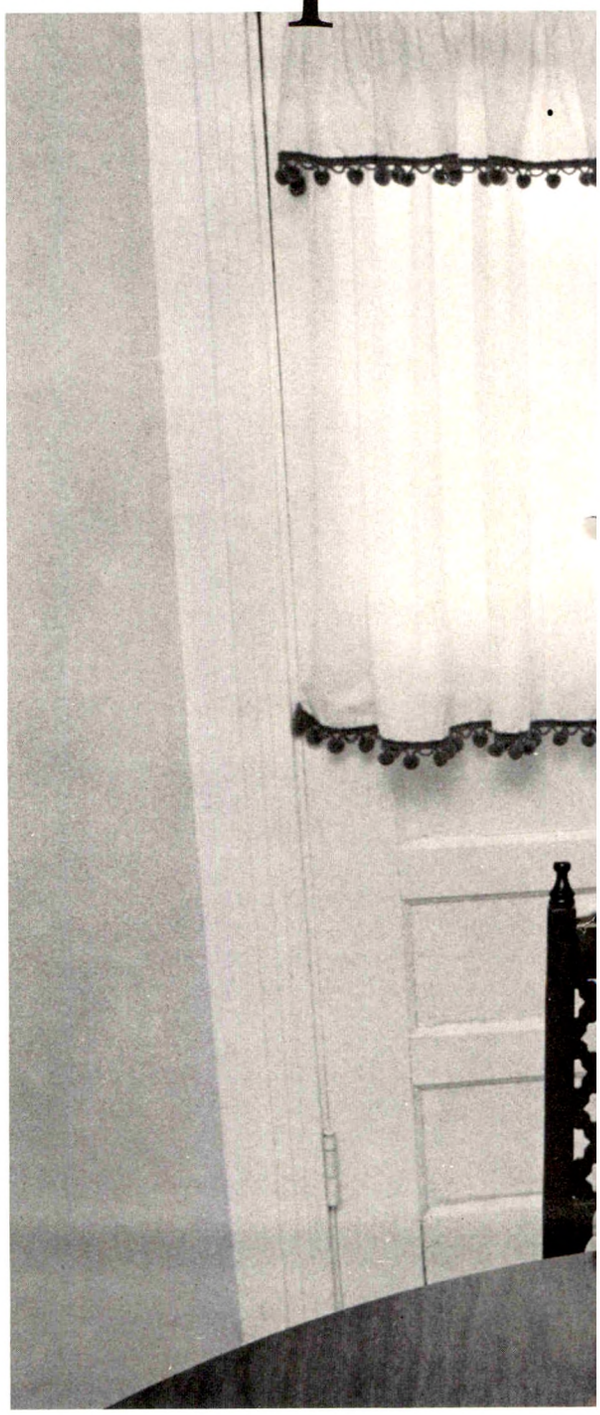
Judy Barrett, Housewife
Pittston, Pennsylvania
Age 22, married 6 years, 2 children
Cycle experience with Zorane 1/50: 8

the majority

"I don't seem to gain much weight with the pill and I don't get sick. I take it at night just before I go to bed... then I sleep right through. And I don't get sick."

From real life...

A Woman satisfied her oral contraceptive



Among the reasons patients remain satisfied: Withdrawal bleeding is usually regular and there is usually no undue discomfort

ZORANE 1/50 is a highly effective and generally well-tolerated oral contraceptive—providing the estrogen content and progestin:estrogen ratio that appears to be highly acceptable to the majority of women.

From clinical studies: In a study population of 1156 women, 25,983 cycles, the incidence was 2.2% for amenorrhea and 2.0% for dysmenorrhea. Actual patient dropout rate* was 1.1% (13 subjects) for amenorrhea and 0.2% (2 subjects) for dysmenorrhea. Overall patient dropout rate was 14.4% due to all adverse reactions. Serious reactions, as well as minor adverse reactions, have been reported following the use of all oral contraceptives. Before prescribing, the complete product information should be consulted for *special note, contraindications, warnings, precautions* and *adverse reactions*. Thereafter, the physician should remain alert to the earliest manifestations of any serious disorder. Should any occur or be suspected, the oral contraceptive should be immediately discontinued.

The woman profiled here is an actual ZORANE 1/50 user. However, not all women can be expected to have the same tolerance for side effects, should any occur. (Data on file, Lederle Laboratories)

*Dropout rates are based on total patient population (1,156); incidence reflects only those patients who completed one or more cycles (956).

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Mrs. Linda McKinnon, Housewife
Nederland, Texas
Age 25, married 7 years, 2 children
Cycle experience with Zorane 1/50: 16

the majority

"You always know when you're going to start…I love knowing that… you can always plan…I think it's great. And, not having any cramps, too, because I used to have cramps."

From real life...

A Woman satisfied her oral contraceptive



One reason for long-term patient satisfaction: Weight gain usually not a problem

ZORANE 1/50 is a highly effective and generally well-tolerated oral contraceptive—providing the estrogen content and progestin:estrogen ratio that appears to be highly acceptable to the majority of women.

From clinical studies: In a study population of 1156 women, 25,983 cycles, the incidence of weight gain was 0.4%. Actual patient dropout rate* due to weight gain was 2.0% (23 subjects). Overall patient dropout rate was 14.4% due to all adverse reactions. Serious reactions, as well as minor adverse reactions, have been reported following the use of all oral contraceptives. Before, prescribing, the complete product information should be consulted for *special note, contraindications, warnings, precautions* and *adverse reactions*. Thereafter, the physician should remain alert to the earliest manifestations of any serious disorder. Should any occur or be suspected, the oral contraceptive should be immediately discontinued.

The woman profiled here is an actual ZORANE 1/50 user. However, not all women can be expected to have the same tolerance for side effects, should any occur. (Data on file, Lederle Laboratories)

*Dropout rates are based on total patient population (1,156); incidence reflects only those patients who completed one or more cycles (956).

Zorane® 1/50

Norethindrone Acetate, 1 mg and Ethinyl Estradiol, 50 mcg
Oral Contraceptive Tablets

Progestin/Estrogen content balanced fo

See last page of this advertisement for brief summary of prescribing information.

with



Alice Eichenberger, High School
Business Teacher, Louisville, Kentucky
Age 28, married 7 years, no children
Cycle experience with Zorane 1/50: 13

the majority

"I've fluctuated between 115 and 120...and have had no appreciable weight gain...since I've been on Zorane."

Zorane® 1/50

Progestin/Estrogen content balanced for the majority

♀ Norethindrone Acetate, 1 mg and Ethinyl Estradiol, 50 mcg

Oral Contraceptive Tablets

Also available from Lederle

Zorane 1.5/30 Norethindrone Acetate, 1.5 mg, and Ethinyl Estradiol, 30 mcg.

Zorane 1/20 Norethindrone Acetate, 1 mg, and Ethinyl Estradiol, 20 mcg.

Special Note: Oral contraceptives have been marketed in the United States since 1960. Reported pregnancy rates vary from product to product. The effectiveness of sequential products appears to be somewhat lower than that of combination products. Both types provide almost completely effective contraception. The dropout rate for medical reasons, as observed in clinical trials, with ZORANE 1.5/30 appears to be the same as that of higher dose combination products. The dropout rate due to irregular bleeding was somewhat higher, but dropouts were equally split between irregular bleeding and other medical reasons attributable to the drug. (ZORANE 1/20 only: The effectiveness is somewhat lower than that of the higher dose combination products. Both provide almost completely effective contraception. Dropout rate for medical reasons appears to be somewhat higher than with higher dose combination products, due particularly to irregular bleeding.) An increased risk of thromboembolic disease associated with use of hormonal contraceptives has now been shown in studies conducted in both Great Britain and the United States. Other risks, such as those of elevated blood pressure, liver disease, reduced tolerance to carbohydrates, have not been quantitated with precision. Long-term administration of both natural and synthetic estrogens in subprimate animal species in multiples of the human dose increases the frequency for some animal carcinomas. These data cannot be transposed directly to man. Possible carcinogenicity due to the estrogens can neither be affirmed nor refuted at this time. Close clinical surveillance of all women taking oral contraceptives must be continued.

Indications: For control of conception.

Contraindications: Thrombophlebitis, thromboembolic disorders, cerebral apoplexy, or a past history of these conditions; markedly impaired liver function; known or suspected carcinoma of the breast; known or suspected estrogen-dependent neoplasia; undiagnosed abnormal genital bleeding; pregnancy.

Warnings: If early signs of thrombotic disorders (thrombophlebitis, cerebrovascular disorders, pulmonary embolism, retinal thrombosis) occur or are suspected, discontinue use of drug immediately. (Studies indicated a higher risk of these conditions with use of these drugs, not persisting after drug stoppage and not enhanced by long-term use.[1-4]) If sudden partial or complete loss of vision occurs, or if there is sudden onset of proptosis, diplopia or migraine; or if examination reveals papilledema or retinal vascular lesions, discontinue medication. Safety of use in pregnancy has not been established, therefore rule out pregnancy in any patient who has missed two consecutive periods before continuing contraceptive regimen; if prescribed schedule has not been adhered to, consider possibility of pregnancy with first missed period. A small fraction of hormonal agents has been identified in the milk of mothers receiving these drugs; long-range effect to the nursing infant has not yet been determined.

Precautions: Pretreatment and periodic physical examinations of breasts and pelvic organs should include Papanicolaou smear. (Estrogens have produced tumors, some malignant, in 5 species of subprimate animals.) If endocrine and liver function tests are abnormal in patients taking these drugs, repeat tests after drug withdrawal for 2 months. With use of these drugs, pre-existing uterine fibromyomata may increase in size; and some conditions (*i.e.*, epilepsy, migraine, asthma, cardiac or renal dysfunction) may be influenced by fluid retention; observe carefully for such. With use of ZORANE 1.5/30 or ZORANE 1/20, alteration in menstrual patterns may be induced. Since the amount and duration of flow and cycle length may vary, this must be considered in attempting to determine if cause of bleeding is organic. (ZORANE 1/50 only: In breakthrough bleeding or all cases of irregular bleeding per vaginam, keep in mind nonfunctional causes and take adequate diagnostic measures.) With history of psychic depression, discontinue drug if depression recurs to a serious degree. Possible influence of prolonged ZORANE therapy on pituitary, ovarian, adrenal, hepatic or uterine function awaits further study. A decrease in glucose tolerance has been seen in some patients on oral contraceptives; carefully observe diabetics on such therapy. Patient age is no absolute limiting factor, although these drugs may mask onset of climacteric. The pathologist should be advised of ZORANE therapy when relevant specimens are submitted. Susceptible women may have an increase in blood pressure following use of contraceptive steroids.

Adverse Reactions Observed in Patients Receiving Contraceptives: Statistically significant association demonstrated between use of oral contraceptives and: thrombophlebitis, pulmonary embolism, cerebral thrombosis. Suggestive evidence, neither confirmed nor refuted, between use and neuro-ocular lesions (*e.g.*, retinal thrombosis, optic neuritis). Known to occur: nausea, vomiting, G.I. symptoms (abdominal cramps, bloating), breakthrough bleeding, spotting, change in menstrual flow, amenorrhea during and after treatment, edema, chloasma or melasma, breast changes (tenderness, enlargement, secretion), weight change (increase or decrease), changes in cervical erosion and secretions, suppression of lactation when given immediately post-partum, cholestatic jaundice, migraine, allergic rash, rise in blood pressure in susceptible women, mental depression. Reported association with use, neither confirmed nor refuted: anovulation post treatment, premenstrual-like syndrome, changes in libido, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, fatigue, backache, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, itching. *May Alter Laboratory Results in:* hepatic function (increased sulfobromophthalein retention and other tests), coagulation tests (increase in prothrombin, Factors VII, VIII, IX, X), thyroid function (increase in PBI_1, and butanol extractable protein bound iodine, and decrease in T^3 uptake values), metyrapone test, pregnanediol determination.

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FETUS, PLACENTA, AND NEWBORN

Diagnosis of placental sulfatase deficiency

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Placental sulfatase deficiency has been found in four pregnancies (cases 1 to 4) with inappropriately low levels of urinary estriol excretion (less than 1.3 mg. per day near term gestation) associated with healthy neonates. The basis of the diagnosis in these cases was the greatly limited capacities for hydrolysis of ^{14}C-dehydroepiandrosterone sulfate (DHA-S) and ^{3}H-estrone sulfate (0.2 μCi each) to the free steroids during incubation of placental homogenates. Placental aromatase activities in vitro for free DHA and the concentrations of appropriate estrogen precursors in cord blood were normal or elevated. The defect was diagnosed prenatally in two of these cases on the basis of failure to increase the maternal excretion of urinary estriol (0.6 to 0.7 and 1.3 to 1.3 mg. per day, respectively) following acute instillation of DHA-S (250 mg.) into the amniotic fluid and on normal levels of estrogen precursors in cord blood. In comparison, a twofold increase in maternal estriol excretion was observed after infusing DHA-S into the amniotic cavity of a "high-risk" pregnancy having normal sulfatase and aromatase activities in vitro (case 5). These enzyme activities were also found to be similarly normal in another placenta from an undergrown fetus (case 6) and in six normal placentas. The clinical features of these pregnancies, the first ones described from the western hemisphere, are similar to reported cases: the newborn progeny are healthy males who appear to be developing normally. The prenatal diagnosis of the sex-specific placental enzyme defect has been made possible by the use of an intra-amniotic DHA-S loading test.

PARADOXICAL clinical discrepancies between urinary estriol excretion and fetal health may result from a variety of causes including incorrect collections, interfering medications, and a host of pathologic factors of fetal, placental, or maternal origin. Extremely low estriol excretion rates have been associated with fetal death in utero, adrenal hypoplasia, anencephaly, fetal hepatitis, placental sulfatase deficiency, and severe maternal renal disease. Recently seven cases of placental sulfatase deficiency have been reported in which the placental enzyme system hydrolyzing steroid 3β-sulfates has been inactive in in vitro incubations[1–4] or perfusion[5] of the placenta.

Our recent observation of pregnant patients with extremely low estriol excretion rates near term gestation (less than 1.3 mg. per day) associated with healthy newborn infants prompted us to initiate an investigation into the metabolism in vitro of androgen and estrogen sulfoconjugates by placentas from these pregnancies and to quantitate appropriate estrogen precursors in the umbilical cord blood of these infants.

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Table I. Summary of clinical details of the pregnancies

Patient	Parity	Gestational weeks	Urinary steroids (mg./day)		Maternal-fetal complications	Gestational weeks at delivery	Fetus		Placental Weight (Gm.)	Mode of delivery
			Estriol	Estrone			Sex	Weight (Gm.)		
Deficient placental sulfatase activity:										
1. V. B.	1	42	0.5	—	Prolonged pregnancy; viral hepatitis (41 wk.)	43	M	3,855	555	Oxytocin induction; forceps
		42	1.2	—						
2. D. F.	1	40	0.8	—	None	40	M	2,900	550	Oxytocin induction; forceps
		40	0.8	—						
		40	1.1	0.08						
3. R. H.	2	35	0.6	—	Intrapartum fetal distress; history of prior intrauterine death (36 wk.)	38	M	2,966	510	Spontaneous labor; cesarean section for fetal distress
		37	0.7*	0.7						
		37	0.6*	0.7*						
		37	0.7*	—						
4. L. Y.	1	44	1.3	—	Prolonged pregnancy	44	M	2,778	520	Oxytocin induction; cesarean section
		44	1.3*	—						
		44	1.3*	—						
Normal placental sulfatase activity:										
5. R. R.	2	36	7.5	—	Suspected fetal adrenal hypofunction; Male siblings, congenital adrenal hypoplasia	40	F	3,438	510	Spontaneous labor and delivery
		37	8.2	—						
		38	11.6	1.3						
		38	17.5*	2.7*						
		38	20.8*	—						
6. S. H.	2	38	3.0	—	Intrapartum pre-eclampsia; intrauterine growth retardation	40	M	2,608	455	Oxytocin induction; forceps
		38	2.0	—						

*After intra-amniotic instillation of DHA sulfate (250 mg.).

The intrauterine injection of DHA-S was also carried out to evaluate the capacity of the intact fetoplacental system to utilize this precursor for the production of estrogenic urinary metabolites.

Materials and methods

Clinical data. Pertinent clinical data of six pregnancies, four of them associated with very low levels of urinary estriol excretion, are summarized in Table I. Repeated examination of maternal urine specimens from V. B., (case 1), a 23-year-old primigravida with prolonged pregnancy (43 weeks), and D. F. (case 2), a 19-year-old primigravida with an otherwise normal pregnancy, revealed extremely low values of estriol (less than 1.2 mg. per day). Their infants, both males weighing 3,855 and 2,900 grams, respectively, were delivered in excellent condition following oxytocin induction of labor. R. H. (case 3), was a 21-year-old patient with a previous history of intrauterine fetal death at term gestation. In this pregnancy the urinary estriol excretion after 35 weeks' gestation was repeatedly 0.7 mg. per day or less. Cesarean section at 38 weeks' gestation was performed for intrapartum fetal distress with delivery of a healthy male infant weighing 2,966 grams (Apgar score, 9). L. Y. (case 4), a 19-year-old primigravida with prolonged pregnancy (44 weeks) had very low urinary estriol (1.3 mg. per day) and was delivered of a male infant weighing 2,778 grams by cesarean section following a short attempt at induction of labor. R. R. (case 5) was a 26-year-old patient having a clinically normal pregnancy apart from low urinary estriol (7.5 to 11.5 mg. per day near term gestation). She was delivered of a healthy female infant weighing 3,438 grams with an Apgar score of 8; this child is healthy at 3 years of age. A 2-year-old sibling had been treated regularly with cortisone (20 mg. per day) for congenital adrenal hypoplasia. Intra-amniotic instillation of DHA-S was carried out (3 to 4 days before delivery) in cases 3, 4, and 5, with subsequent measurement of urinary estrogen excretion and measurement of plasma androgens in umbilical cord blood. In case 6 (S. H.) a low urinary estriol (2.0 to 3.0 mg. per day) was associated with intrauterine growth retardation near term and intrapartum pre-eclampsia; a dysmature male infant weighing 2,606 grams with an Apgar score of 9 was delivered after oxytocin induction of labor at 40 weeks' gestation.

Steroid assays in blood and urine. Urinary estriol

Table II. Plasma concentrations of C_{19}-Δ^5-3β-OH-steroids in umbilical cord blood (μg/100 ml. of plasma)*

Patient	*DHA*†	*16α-OH-DHA*	*16-keto-A⁵-diol*	*A⁵-triol*
Deficient placental sulfatase activity:				
1. V. B.	28.3	253.9	23.7	866.8
3. R. H.†	30.7	220.9	22.7	1,248.5
Normal placental sulfatase activity:				
4. R. R.†	28.5	94.7	10.5	146.9
Normal pregnancies:				
Mean	40.4	207.8	30.9	70.5
± S.D., n = 7	± 13.8	± 54.5	± 9.4	± 10.9

*Values are uncorrected for procedure losses and represent the mean of duplicate determinations for each.

†DHA (3β-hydroxyandrost-5-en-17-one); 16α-OH-DHA (3β,16α-dihydroxyandrost-5-en-17-one); 16-keto-A⁵-diol (3β,17β-dihydroxyandrost-5-en-16-one); and A⁵-triol (androst-5-ene-3β,16α,17β-triol); 3-4 days after DHA-S intra-amniotic instillation (250 mg.).

was measured by gas liquid chromatography (GLC) as described previously.[6] Urinary ring Dα-ketolic estrogens were quantitated spectrophotometrically by Hobkirk and Nielsen.[7] DHA, 3β,16α-dihydroxyandrost-5-en-17-one (16α-OH-DHA), 3β,17β-dihydroxyandrost-5-en-16-one (16-keto-A⁵-diol), and androst-5-ene-3β, 16α,17β-triol (A⁵-triol) in umbilical cord blood were measured by GLC as the trimethysilyl ether derivatives with the use of a 3 per cent XE-60 column[8, 9] after ethylacetate solvolysis[10] of the samples for the determination of plasma steroid sulfates. Mean recovery of these steroids through the procedure prior to GLC was 73.5 per cent. The values (in micrograms per 100 ml. of plasma) are uncorrected for procedural losses.

Placental enzymatic activities. ^{14}C-4-dehydroepiandrosterone sulfate (^{14}C-DHA-S, specific activity 42.0 mCi per mmole), ^{14}C-4-DHA (specific activity 58.8 mCi per mmole), and ^{3}H-estrone sulfate (specific activity 2.85 Ci per mmole) were purified by thin-layer chromatography (TLC) in at least two different systems before use. Radioactivity was measured in a Packard Tri-Carb liquid scintillation spectrometer (Model 3320) with a counting efficiency of 88.8 per cent for ^{14}C and 53.9 per cent for ^{3}H. Adequate counts were accumulated to include 95 per cent confidence limits.

Placentas obtained after delivery were immediately frozen and stored at −20° C until analysis. Placental tissue from normal pregnancy was also prepared and stored in a similar manner. The thawed tissue was dissected free of membranes, washed several times in saline to remove blood, and then homogenized in a Waring Blendor. The 20 per cent homogenates in 10 ml. of Tris buffer solution (10 mM., pH 7.4 for DHA-S and DHA and pH 6.8 for estrone sulfate) containing 1 Gm. of wet tissue were incubated with 0.2 μCi of radioactive substrates at 37° C for 60 minutes under an atmosphere of 95 per cent oxygen and 5 per cent carbon dioxide with continuous shaking in a Dubnoff incubator. The reaction was stopped by adding 5 ml. of ether and cooling in an ice bath, and the preparation was frozen until analysis. The incubation mixtures were extracted three times with two volumes of ether. The ether extracts containing the "free" fraction were evaporated, and the residue was dissolved in 60 ml. of benzene-petroleum ether (1:1) (BPE) solution from which the "phenolic" fraction was extracted two times with 20 ml. of 0.4N sodium hydroxide according to the Brown[11] method. Subsequently, the "phenolic" fraction was separated by TLC in the solvent system cyclohexane:ethylacetate (1:1) into radioactive peak areas corresponding to standard estrone (E_1), estradiol-17β (E_2), and estriol (E_3). These were eluted and further purified by additional TLC in the system chloroform:methanol (19:1). To the remainder of the BPE containing the "neutral" fraction, three volumes of ethanol were added, followed by filtration and drying. The residue was extracted with 30 ml. of secondary butanol for the determination of the "sulfate" fraction. The specific placental sulfatase activity (per cent per gram of wet tissue per hour) was calculated from the per cent of hydrolysis of the added radioactive steroid sulfate to the free steroids, and the aromatase activity, the rate (per cent) of transformation to radioactive estrone and estradiol from ^{14}C-DHA. Radiochemical homogeneity of the metabolites formed was based on the chromatographic properties of the radioactive products and the respective authentic standards, and recrystallization to constant specific activity.

Infusion of DHA-S. Dehydroepiandrosterone sulfate (Steraloids, Inc., New York, New York) was purified by recrystallization with aqueous methanol before use. Then 250 mg. of DHA-S dissolved in 14 ml. of 50 per cent ethanol in sterile saline were injected after Millipore filtration into the amniotic fluid of

Table III. In vitro steroid metabolic evidence of placental sulfatase deficiency (per cent distribution of the steroid

	(i) ^{14}C-DHA-S						*(ii) ^{14}C-DHA*				
	"Free"						*"Free"*				
	Neutral	*Phenolic*	*Estrone (E_1)*	*Estradiol (E_2)*	*E_1/E_2*	*"Sulfate"*	*Neutral*	*Phenolic*	*Estrone (E_1)*	*Estradiol (E_2)*	*E_1/E_2*
Deficient placental sulfatase activity:											
1. V. B.	5.2†	1.8	—	—	—	69.6	26.9	61.9	39.9	10.7	3.7
2. D. F.	4.8	1.4	—	—	—	87.6	15.6	54.4	31.6	11.0	2.9
3. R. H.	3.2	0.5	—	—	—	89.7	21.2	50.3	29.0	9.1	3.2
4. L. Y.	5.1	0.7	—	—	—	68.6	27.0	49.2	30.5	10.2	3.0
Normal placental sulfatase activity:											
5. R. R.	43.2	36.9	18.9	7.6	2.5	7.3	14.9	48.2	26.8	13.5	2.0
6. S. H.	50.6	36.9	16.5	5.1	3.2	3.6	34.5	47.1	28.6	10.5	2.7
Normal pregnancies:											
Mean	38.6	38.4	20.3	8.5	2.5	5.8	12.5	54.9	31.7	11.1	2.8
± S.D., n = 6	± 9.4	± 9.2	± 1.4	± 0.7	± 0.2	± 2.3	± 2.5	± 3.9	± 2.8	± 0.8	± 0.2

*The conditions of the duplicate incubations were at 37° C. for 60 minutes of a 1.0 Gm. placental homogenate with 0.2 μCi of (i) ^{14}C-DHA-S, (ii) ^{14}C-DHA (each at pH 7.4), and (iii) ^{3}H-estrone sulfate (pH 6.8). The conversion rate (per cent) to estrone and estradiol-17β was calculated from the radioactivity eluted, the peak areas corresponding to the respective standards on the TLC plate.

†Values are per cent of initial radioactivity (0.2 μCi) isolated in the individual fractions.

R. H. (case 3), L. L. (case 4), and R. R. (case 5) during a 10 minute interval 3 to 4 days prior to delivery.

Results

Maternal urinary estrogen excretion. The estriol excretion rates and clinical data on the six patients are summarized in Table I. In the four patients with placental sulfatase deficiency (cases 1 to 4), the levels of maternal urinary estriol were extremely low (less than 1.3 mg. per day) at the end of pregnancy. The lower limits of excretion for normal pregnancies with our method are 10 mg. per day at 30 weeks' gestation, 15 mg. per day at 35 weeks, and 18 mg. per day at term. Patient 2, with placental sulfatase deficiency, had greatly reduced urinary levels of 16-keto-estradiol-17β, and 16α-OH-estrone, respectively, 0.09 (2.0)* and 0.12 (2.9)* mg. per day.

Plasma concentrations of Δ^5-C_{19}-steroids in umbilical cord blood. DHA, 16α-OH-DHA, 16-keto-A^5-diol, and A^5-triol were measured in cord blood of normal pregnancies (n = 7) and of pregnancies with placental sulfatase deficiency and suspected fetal adrenal hypofunction (Table II). Our normal values for the plasma concentration of these C_{19} steroids were comparable to those reported previously by France,[12] using a similar GLC assay. The levels of A^5-triol in these cases of placental sulfatase deficiency (cases 1 and 3) were significantly higher than those of normal pregnant women, possibly in case 3 because of the DHA instillation; however, DHA, 16α-OH-DHA, and 16-keto-A^5-diol levels were within the normal range. Lower concentrations of these 16-oxygenated androgens were observed in the cord blood, even after instillation of 250 mg. of DHA-S in Patient 4, in whom adrenal hypofunction was suspected because of reduced levels of urinary estriol and a sibling with this defect.

*Mean values in normal term pregnancies.[7]

Placental enzymatic activities. The data obtained from duplicate incubations of placental homogenates with (i)^{14}C-DHA-S, (ii)^{14}C-DHA, and (iii)^{3}H-estrone-sulfate (Table III) indicated greatly reduced conversion rates of the steroid sulfates to the "free" fractions in the placentas of cases 1 to 4, compared with the higher rates of hydrolysis from normal control placentas (n = 6). Approximately 70 to 80 per cent of the initial radioactivity (DHA-S and estrone sulfate) remained in the "sulfate" fraction. In contrast, the hydrolyis of both sulfates and the in vitro conversion of ^{14}C-DHA to estrogen metabolites in the placentas obtained from cases 5 and 6 were identical to those obtained from the normal control placentas (n = 6). The distribution of radioactivity in the "phenolic" fraction isolated after the placental incubations of cases 1 to 4 with ^{14}C-DHA showed no difference from those of normal placental incubations, indicating normal aromatase activity. The "phenolic" fractions were further separated into estrone, estradiol, and estriol with TLC. The radiochemical homogeneity of estrone and estradiol was demonstrated by recrystallization to constant specific activities. The calculated estrone/estradiol ratios, ranging between 2.0 and 3.7 from the four placentas studied, were similar to the

metabolites)*

	(iii) ^{3}H-estrone sulfate				
	"Free"				
"Sulfate"	Phenolic	Estrone (E_1)	Estradiol (E_2)	E_1/E_2	"Sulfate"
0.3	5.1	3.1	1.2	2.6	72.1
0.5	18.2	8.1	3.6	2.2	76.5
0.4	15.6	6.3	2.5	2.6	76.2
0.5	6.8	3.8	1.5	2.4	87.6
1.5	80.3	40.2	19.1	2.2	5.6
1.7	85.8	48.4	20.4	2.4	7.4
0.8 ± 0.6	84.7 ± 6.1	53.8 ± 6.2	21.2 ± 2.9	2.8 ± 0.4	5.2 ± 1.6

normal values (2.8 ± 0.3). No radioactive peak on the TLC plates in any experiment corresponded to authentic estriol.

DHA-S loading test. The intra-amniotic instillation of 250 mg. of DHA-S did not change the rate of maternal excretion of estriol (0.6 to 0.7 mg. in case 3 and 1.3 to 1.3 mg. in case 4 per 24 hours) and estrone (0.7 to 0.7 mg. per day in case 3) by the woman with extremely reduced excretion rates, but antenatal administration of DHA-S into the amniotic fluid of the patient (case 5) with the normal placental sulfatase and aromatase activities in vitro increased the excretory rates from 11.6 to 20.8 mg. per day for estriol and from 1.3 to 2.7 mg. per day for estrone (Fig. 1).

Comment

A sulfatase deficiency has been discovered in placentas from four women having clinically normal pregnancies with extremely low estriol excretion near term. In two of them the defect was diagnosed prenatally on the basis of failure to increase the low levels of maternal urinary estriol following the intra-amniotic infusion of DHA-S. These cases of placental sulfatase deficiency are the first ones described from the Western Hemisphere, although another presumptive case has been mentioned in abstract form.[13] The clinical features of the pregnancies studied are similar to the seven proved and four presumptive cases reported before.[1-5] All four of our infants were also healthy males who are developing normally. On the basis of 10^{1-5} consecutive male progeny, the condition appears to be sex-linked ($p < 0.001$, nonparametric tests) and suggestive of an X-linked recessive inheritance. Genetic studies in families with a pregnancy having a placental sulfatase deficiency will be valuable to explain the fundamental mechanisms causing the enzyme defect. Labor occurred spontaneously at term gestation in one pregnancy, was induced at term in another, and was delayed beyond 43 weeks' gestation in two cases, one of which also responded to oxytocin induction. Prior observers have commented that prolonged pregnancy and unsuccessful attempts at induction of labor characterize this group of patients, especially if primigravid. Our experience suggests that induction of labor is successful and therefore advisable.

The antepartum diagnosis of a placental sulfatase deficiency would be helpful for the clinician trying to interpret the meaning of extremely low urinary estrogen excretion rates in women not taking the various drugs known to depress estriol excretion. Fetal death and anencephaly, the conditions most likely to account for such findings, usually can be diagnosed by several procedures but differentiation between fetal adrenal hypoplasia and placental sulfatase deficiency is more difficult. The diagnosis can be ascertained by demonstrating a differential response in urinary estriol excretion following administration of the steroid sulfates into these patients. The DHA-S loading provides a sensitive test of the fetoplacental function.[14-16] The role of fetal[17, 18] rather than maternal DHA-S[19] in the production of urinary estriol during pregnancy is well established. It is probable that the very small amounts of estriol excreted by these women were from maternal 16α-OH-DHA-S via its hydrolysis in the maternal liver entering the placenta and being metabolized to estriol. Infusion of precursor DHA-S into the fetal compartment rather than in the maternal circulation of normal pregnancy produces a much greater rise in the estriol production.[20, 21] Therefore, we infused the steroid sulfates into the amniotic fluid. A twofold increase of maternal estriol excretion was observed after infusing DHA-S into amniotic fluid of a "high-risk" pregnancy having normal placental sulfatase and aromatase activities in vitro. Patients 3 and 4 had no significant change in estrogen excretion after DHA-S loading and we therefore interpret prenatally that the placentas were deficient in sulfatase activity.

Previous workers have interpreted, on the basis of normal cord blood levels of DHA and 16α-OH-DHA, that estrogen precursors do not accumulate in the fetal circulation of sulfatase-deficient pregnancies, and therefore that the fetus has a feedback mechanism controlling production of C_{19} steroid sulfates.[3, 4] Our finding of greatly increased levels of A^5-triol in cord

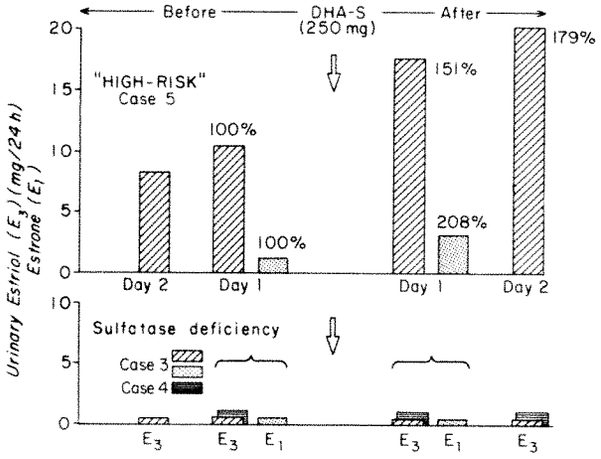


Fig. 1. Estrogen excretion before and after intra-amniotic instillation of 250 mg. of DHA-S (dehydroepiandrosterone sulfate) in a "high-risk" pregnancy (case 5) and two patients (cases 3 and 4) with placental sulfatase deficiency. The numbers above the columns represent the percentage of values just prior to the instillation.

blood, particularly following instillation of DHA-S into the amniotic fluid, suggest that this end product of DHA (or its sulfate secreted from the fetal adrenal) accumulates in the fetal circulation if the placental sulfatase activity is lacking. One is also tempted to interpret on the basis of normal cord blood concentrations of 16-keto-A^5-diol in these pregnancies, that this compound is not a direct precursor of estriol in term pregnancies. These data are consistent with the conclusions based upon perfusion studies in midpregnancies[22, 23] and the demonstration of the fetal hepatic production of 16-keto-A^5-diol.[24]

Our attention was drawn to these cases by a low urinary estriol excretion and it was possible to establish the diagnosis antepartum in two cases and postpartum in two additional cases. Instillation of DHA-S (nonradioactive) into the amniotic fluid and observation of changes of urinary estriol excretion is a simple procedure with little apparent associated hazard. With a relative contraindication of using radioactive substrates during normal pregnancy, alternate methods should be sought for accessing placental function since studies relying on maternal intravenous administration of steroids have not been particularly helpful in predicting pathology of the fetus or placenta.[25, 26]

We are indebted to Drs. D. Cox (case 1), our house staff (case 2), D. M. Barton (case 3), L. Brewer (case 4), N. R. M. Buist (case 5), and J. H. Mattox (case 6) for their kind help in supplying us with the clinical materials, and Dr. R. Hobkirk for analysis of urinary Ring Dα-ketolic estrogens. The valuable technical assistance of Mrs. Pearl Numkung and the typing of the manuscript by Mrs. Ann Dow are also greatly appreciated.

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Histologic characteristics of cells cultured from rat placental tissue

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Laboratory animals, especially rats, have provided a comparative model for extensive research on maternal-fetal relationships. To identify individual placental cells for subsequent immunologic analysis, cultures of rat placentas were studied. Five cell types were observed, including: two histologically distinct giant cells, mononucleate and multinucleate, apparently formed by amitosis and fusion, respectively; small round cells; polygonal cells, probably cytotrophoblasts; and fibroblasts. Of particular interest are the small round cells which are phagocytic and morphologically similar to macrophages, cells known to be important in the immune response.

DESPITE intensive investigations in many species, the precise sequence and biologic significance of cellular changes occurring in the placenta during pregnancy remain obscure. Least understood is the nature of protection afforded by the intact placental structure against possibly disastrous immunologic consequences that could result from maternal exposure to paternal histocompatibility antigens throughout gestation.

Tissue-culture techniques have been applied to study the nature of these cellular changes. In numerous studies of cultures of human placental cells, three morphologically distinct cell types have been found: (1) epithelioid cells, (2) multinucleate cells, and (3) fibroblasts.[10] Studies of mouse placental cells in culture have indicated, in addition, a fourth cell type, a mononucleate giant cell.[12] The developmental sequences, interactions, and physiologic characteristics of the individual hemochorial placental cell types, in particular the outermost, immunologically significant, mononuclear (rodent) or multinucleate syncitial (human) giant cells, are still unresolved. One problem has been the difficulty in defining serologically and immunogenically the individual placental components.

The studies that follow were initiated to investigate the cellular heterogeneity of rat placenta and provide a basis for serologic studies of each cell type.

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Methods and material

Tissue culture preparation. Cells were obtained from the placentas of 12- 13-day-old embryos of the DA and BN inbred rat strains. The uterus was removed aseptically, washed several times in phosphate-buffered saline (PBS), opened and washed again, and then placed in calcium- and magnesium-free phosphate-buffered saline (CMF-PBS). Each embryo and placenta was "shelled out" intact, placed in fresh CMF-PBS, and carefully separated from one another. The outermost placental tissue, consisting primarily of the giant-cell layer and the cytotrophoblast cells, was selected and placed in fresh CMF-PBS, minced with scissors, and washed again. Small pieces were then transferred to a 25 ml. Erlenmeyer flask to which 10 ml. of 0.1 per cent tryspin (Flow Laboratories) was added. Trypsinization was performed with constant stirring at 37° C. The first supernatant, containing mostly red blood cells, was removed and discarded after 10 minutes. Subsequent cell suspensions were

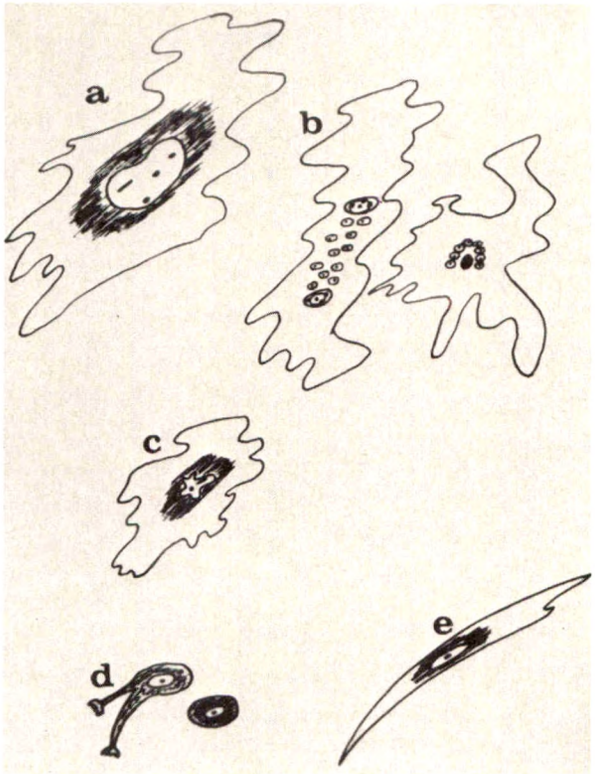


Fig. 1. In vitro cell types cultured from rat placental tissues; *(a)* mononuclear giant cells, *(b)* multinucleate giant cell, *(c)* polygonal cells, *(d)* small round cell, and *(e)* fibroblast.

collected, centrifuged, split into equal fractions, and placed in sterile 35 mm. plastic Petri dishes (Falcon Plastics) containing sterile coverslips. Autopow MEM (Flow Laboratories) supplemented with amino acids, L-glutamine, and 20 per cent heat-inactivated fetal calf serum was used as a culture medium. The cell cultures were maintained in a 37° C. incubator in a humid 5 per cent CO_2 and 95 per cent air atmosphere.

Radioactive thymidine labeling. For continuous labeling, the cultures were maintained in medium containing tritiated thymidine (New England Nuclear, specific activity 6.7 Ci/mM), at a concentration of 0.05 or 0.1 μCi per milliliter, and were harvested for observation at various times after addition of the label.

Coverslips from the cell cultures were rinsed in PBS and fixed in Carnoy's I solution. After drying, the coverslips with the cells on the upper side were mounted on a gelatinized slide and, 24 hours later, were covered with Kodak Ar. 10 autoradiography stripping film. The slides were dried and stored at 4° C. in sealed slide boxes. After a 10 to 14 day exposure, the slides were developed and fixed. The cells were stained with May-Grünwald Giemsa stain.

Fixation and staining. Coverslips were fixed in 95 per cent ethanol or 10 per cent phosphate-buffered formalin. Staining was done with May-Grünwald Giemsa or hematoxylin and eosin.

Microscopy. The cells and tissues were examined with a Leitz Ortholux microscope. A Leitz automatic camera and Kodak Tri-X (ASA 400) or Ektachrome (ASA 160) film were used for photography.

Results

Heterogeneity of placental cell cultures. Daily observation of the cultures of 12- 13-day-old rat placentas permitted identification of five morphologically distinct cell types: (1) mononuclear giant cells, (2) multinucleate giant cells, (3) large polygonal cells, (4) small round cells, and (5) fibroblast-like cells (Fig. 1). Their morphologic characteristics and developmental changes under culture conditions were as follows:

Mononuclear giant cells. These measured up to 370 μ at the broadest dimension with a nuclear diameter of up to 115 μ (Fig. 2, *A*). The cytoplasm, which exhibited a characteristic deep blue staining, often contained numerous vacuoles of various sizes. Some cells contained more than one nucleus, and occasionally bizarre nuclear forms were observed. Nucleoli were usually two to five in number and were usually very large, round, and dense. Infrequently, a rod-shaped nucleolus was seen (Fig. 2, *A*). In culture, the mononuclear giant cells were often found clumped with other types of cells. The relative numbers of mononuclear giant cells in these cultures showed no increase with time and began to decrease after approximately 9 days in culture. This decrease was usually preceded by extensive vacuolarization both the cytoplasm and the nucleus of these cells. Eventually the nucleus and the cytoplasm became poorly delineated and ghostlike. By 15 days they had usually disappeared from the cultures.

Multinucleate giant cells. These cells, which were up to 200 μ in size, contained anywhere from two to 100 nuclei (Fig. 2, *B*). Although some variability of nuclear size was observed, small round nuclei with one to three nucleoli predominated. Although the number of multinucleate giant cells varied from culture to culture, they usually constituted approximately 10 per cent of the cell population. Often the cells were found adjacent to mononuclear giant cells. Despite the fact that no mitotic figures were seen in these cells, the number of nuclei within them increased until the cells disappeared from the cultures at 9 to 13 days.

Polygonal cells. The large polygonal (or epithelioid) cells were the predominant type, constituting 60 to 70 per cent of the placental cultures, after 9 to 16 days (Fig. 3). Unlike the mononucleate giant cells, these cells, which usually were about 80 μ in size, but

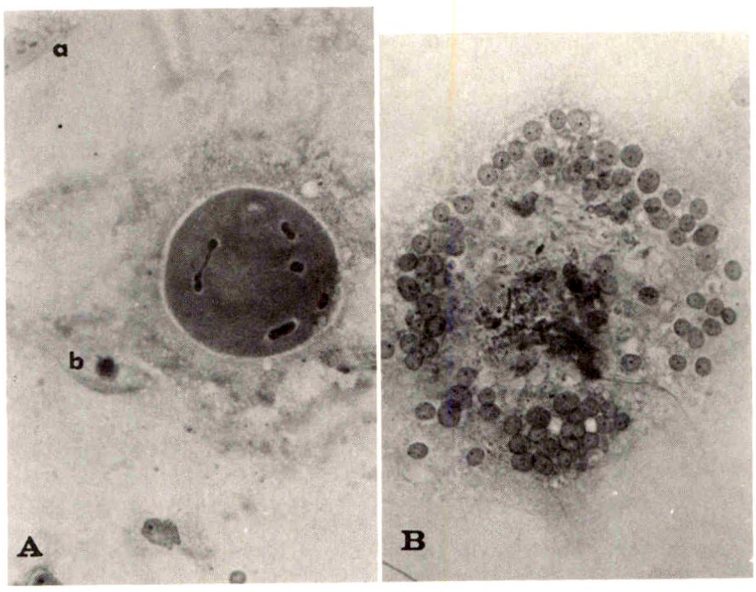


Fig. 2. Mononuclear *(A)* and multinuclear *(B)* giant cells. Note polygonal cell *(a)* and small round cell *(b)*. (×300.)

occasionally reaching 240 μ in broadest dimension, stained poorly. The cells were usually mononucleate, but occasionally two nuclei were observed. The nuclei, as large as those of fibroblasts, were oval, with one to three nucleoli; mitotic figures were frequently seen. Many aberrant nuclear formations, such as budding and ring structures, occurred. Although the cells became confluent between 9 and 16 days in several cultures, they were very fragile and numerous attempts at transferring them were unsuccessful. At about 14 to 16 days, the fibroblasts in the culture began to advance and overgrow these cells.

Small round cells. These cells had a cytoplasm that stained dark blue and a small, round nucleus (Fig. 3). The size of these cells varied greatly, the smallest being 12 to 14 μ in diameter and the largest, 50 to 60 μ. They were scattered throughout the cultures, frequently lying on or within halo-like areas in the cytoplasm of fibroblastic or polygonal cells. Occasionally, fine filamentous pseudopodia were seen extending from them. After a few days in culture many of these cells had enlarged to three or four times their original size, but retained their dark-staining characteristics. Some of the cells persisted during the lifetime of the cultures and could be successfully transferred along with the fibroblastic cells that eventually overgrew the cultures. Phagocytic activity by these cells was observed in several placental cultures after accidental bacterial contamination; the small round cells contained many bacteria (Fig. 4). The other four cell types did not exhibit phagocytic capability.

Fibroblasts. Initially fibroblast cells were rare in all the placental cultures, but their numbers gradually increased and after 14 to 16 days they were the predominant cell. They exhibited morphologically uniform spindle-like characteristics. When they became the predominant cell type, they developed into complete monolayers. A few of these fibroblast cultures were transferred successfully as many as three times.

In addition to these five morphologically distinct cell types, there were some cells in all cultures with intermediate morphologic characteristics that precluded definitely placing them in one of these five categories.

Thymidine labeling of placental cultures. When the success of the placental cell cultures was assured at 48 hours, medium containing thymidine was added. Coverslips were then removed from the cultures at 12, 24, 36, and 48 hours, and at 4, 7, 10, and 13 days. Uptake occurred in all types of cells, but the time of initial appearance, the relative numbers of cells showing nuclear labeling, and the extent of labeling varied between types (Fig. 5). Those cells known to be rapidly dividing, i.e., polygonal cells and fibroblasts, were most rapidly and extensively labeled. At 24 hours, 40 per cent of the polygonal cells were labeled; by 4 days, this increased to 82 per cent. The peak labeling of 90 per cent of this cell type was reached by 7 days. The percentage remained relatively constant until the fibroblast cells overgrew the culture. At 24 hours 62 per cent of the fibroblast nuclei in the culture were labeled and by 4 days this increased to 92 per cent. In contrast, only

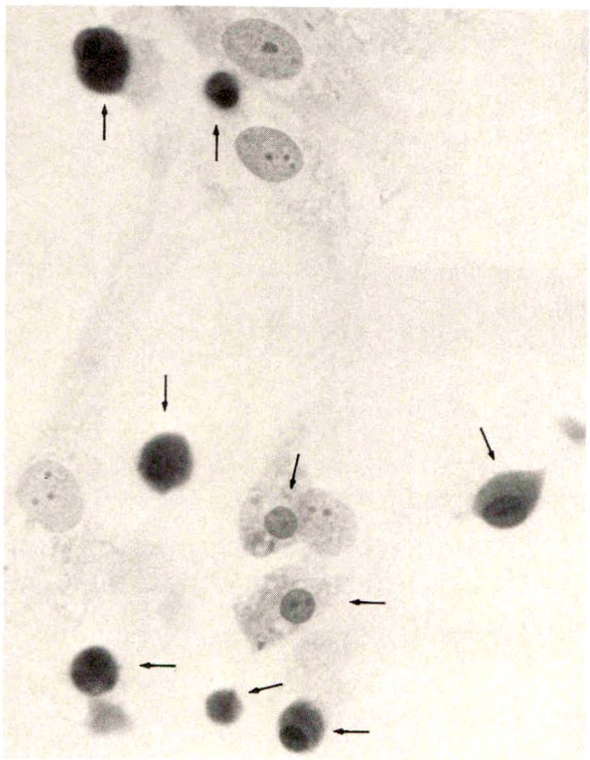


Fig. 3. Round cells of various sizes (arrows) are present in this field. Note also the polygonal cells in the field. The small round cells were found lying on or within the cytoplasm of these cells. (×600.)

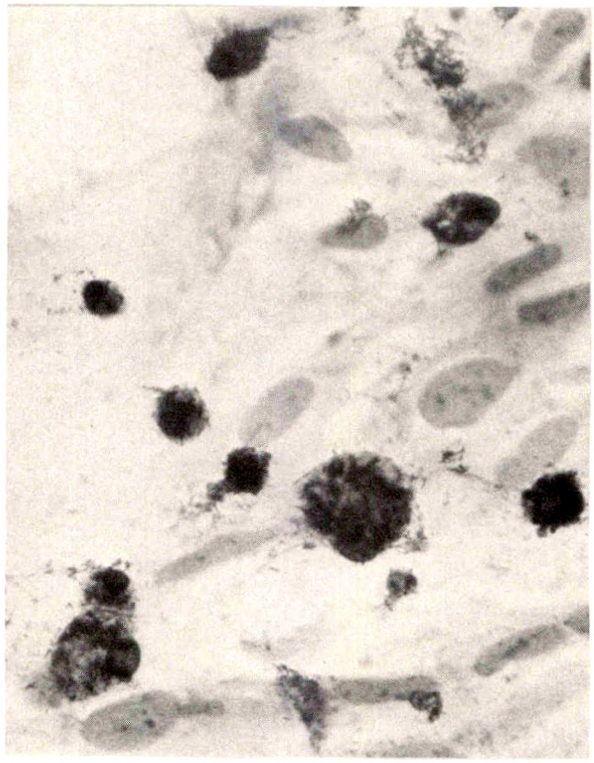


Fig. 4. Phagocytic activity of the round cells indicated by extensive uptake of bacilli. The morphology and size of these phagocytic cells varied greatly. Note absence of bacteria in polygonal cells. (×600.)

6 to 8 per cent of small round cells became labeled.

The two cell types of greatest interest in terms of origin and development, the multinucleate and mononuclear giant cells, exhibited patterns of labeling that distinguished them from the other cells in the cultures.

Mononucleate. Eight per cent of the *mononuclear* giant cells showed thymidine uptake at 12 hours. At 36 hours the figure was unchanged. The label was distributed uniformly across the nucleus. By 4 days the number of cells with labeled nuclei had increased to 28 per cent, a proportion that stayed fairly constant through 7 days. Between cells, the amount of label in the nuclei varied. In the most highly labeled, the density of thymidine uptake, as indicated by the number of grains, was almost comparable to that seen in the polygonal cells and fibroblasts in the cultures.

Multinucleate. The *multinucleate* giant cells, in contrast to the mononucleate cell just described, were unlabeled up to 36 hours after addition of the radioactive thymidine. At 4 days, 6 per cent of these cells contained one or two labeled nuclei mixed with many unlabeled nuclei. Infrequently, some multinucleate cells contained up to three small labeled nuclei and occasionally only a single, large, oval, fibroblast-like, labeled nucleus.

Comment

In recent years comparative studies of human and rodent tissue culture have been utilized to isolate indivual placental cell types and to identify their function. These studies of rat placental cell cultures which were done to differentiate the various cell types for subsequent serologic studies of cell surface antigens indicate a number of previously unreported findings. First, there is a distinct population of *small round cells* closely associated with the placenta. These small, round, intensely stained cells are of particular interest, because their phagocytic activity parallels that of macrophages. Their variable morphologic characteristics are similar to those of blood monocytes or histiocytes, cells known to develop into macrophages, epithelioid cells, and multinucleate giant cells.[17] They may be the counterpart of stellate macrophage-like cells that have been found in human placental cultures.[16] Significantly, the macrophage is an important cellular component of the immune response. A response that is altered in some way to protect the

immunologically incompatible fetus, having antigens inherited from its father, during pregnancy.

The *multinucleate giant cells* present in the cultures apparently are derived from either the small round cells or the placenta-associated maternal tissues. Although multinucleate cells are present initially in the cultures, their number increases, suggesting *de novo* formation. Morphologically these cells appear to be similar to the multinucleate phagocytic cells found in mouse placental cultures.[9, 12] Since these cells are found immediately in primary cultures, they must exist in vivo. Histologic studies suggest their origin, at the twelfth day of gestation, to be from the numerous masses of small round nuclei occurring closely associated with the multinucleate giant cells on the maternal side of the placenta. These masses of nuclei, or "symplasma," were first described at the maternal-fetal junction of the rat placenta by Bridgeman,[4] who suggested that they are maternal in origin and accumulate as the fetal placenta expands and compresses the decidua. Nuclear accumulations, also, are seen at the maternal-fetal junction in many other species.

The presence in cultures of these two cell types, the small round cells and the multinucleate giant cells, and their possible interrelationship raise some interesting questions. If the small round cells are maternal in origin, do they contribute in vivo to the development of the symplasma at the maternal-fetal junction? Alternately, under appropriate culture conditions, do they fuse to become multinucleate giant cells? Are similar macrophage-like cells found associated with the placenta in other species such as the human?

The *thymidine-labeling experiments* provide, to our knowledge, the first tissue-culture information on the DNA synthetic activities of the individual placental cell types. The fact that only 6 to 8 per cent of the small round cells took up thymidine indicates that these cells share another characteristic with macrophages—the tendency not to multiply in culture.[14] The small percentage of labeled nuclei in the multinucleate cells could result from DNA synthesis occurring in only one or two of the nuclei, or, uptake of only one or two of the labeled nuclei, if the cells were being formed by fusion. Our studies do not distinguish between these alternatives, but the presence of an occasional labeled fibroblast-like nucleus within these giant cells suggests that the cells develop by fusion similar to that occurring with the formation of human syncytiotrophoblast[15] or with the development of multinucleate cells in response to foreign bodies.[3, 8]

The most unusual of the cells in terms of cytoplasmic and nuclear characteristics are the *polygonal, or epithelioid, cells.* These appear to be similar to polygonal

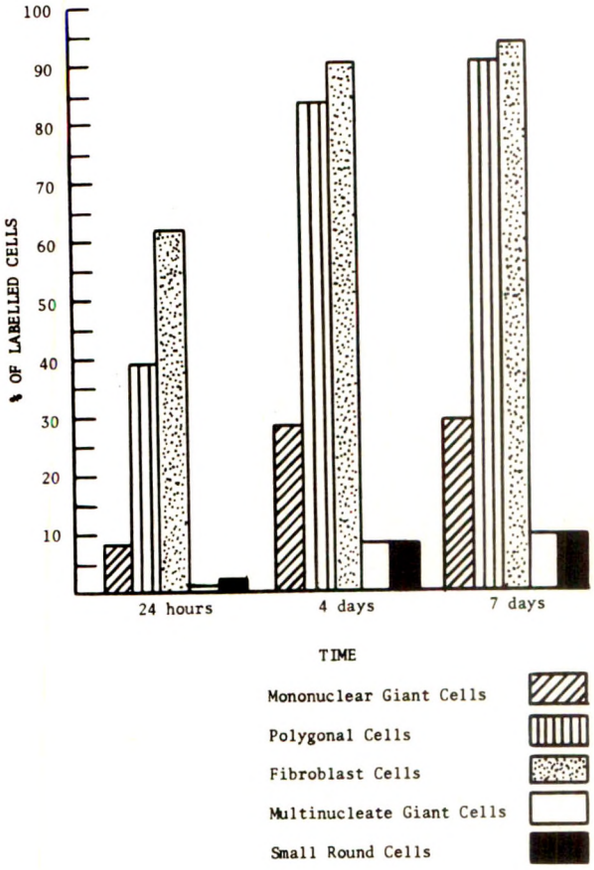


Fig. 5. Thymidine labeling at 24 hours, 4 days, and 7 days of placental cell nuclei in culture.

cells that have been described in mouse cultures,[12] in Millipore chambers containing mouse blastocysts,[5] and in human placental cultures.[10] The comparative morphology suggests that the polygonal cell in vitro is the equivalent of the cytotrophoblast cell in vivo. This would agree with the observation of Fox and Kharkongor,[6] who have shown that a similar human cell in culture has enzyme components of the cytotrophoblast cells. The tissue culture findings of Garancis and associates[7] and Loke and Borland,[10] who reported that similar human polygonal cells actively synthesize chorionic gonadotropin, provide further support for this suggestion.

In contrast to the apparent formation of multinuclear cells, there is no evidence of new *mononuclear giant cells* developing. It is concluded, therefore, that the mononuclear giant cells seen during the 9 to 13 day interval after primary culture had developed in vivo and were placed in culture. The presence and length of survival of the mononuclear giant cells correlates closely with other studies of rodent placental cultures.[9, 12] Histologic in vivo descriptions of these cells indicate

sizes comparable to those found in culture.[1] Our preliminary studies have indicated that no extracellular trophoblast-associated PAS-positive mucoprotein (fibrinoid) is demonstrable under these conditions. This finding concurs with results with mouse placental cells[12] and suggests that cells under these conditions may be useful for immunologic studies of cell surface antigens that otherwise could be masked by fibrinoid.

The tissue-culture observation that mononuclear giant cells incorporate thymidine and/or macromolecular DNA without accompanying cytokinesis has not been reported previously. The uptake of the label as early as 12 hours after exposure without evidence of any mitotic activity correlates with reports of the activity of similar rodent cells in vivo.[11] The density of the thymidine label in the extremely large nuclei of the mononuclear giant cell corresponds to that of the polygonal or fibroblast nuclei in these studies and suggests that mononuclear giant cells are *polyploid* or *polytene*.[13] Other studies, with Feulgen staining and microcytophotometic analysis[2, 18] have actually suggested that the rodent trophoblast giant cell nuclear DNA may be from 100 to 1,000 times the diploid amount. It is difficult to assess the role of the increased DNA, but undoubtedly it is important in the physiologic function of these cells, which are in direct contact with the maternal tissues.

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Intrauterine Development and Fetal Management

Physicians are cordially invited to attend a 2½ day postgraduate course on "Intrauterine Development and Fetal Management" presented by the Johns Hopkins Medical Institutions on Thursday, Friday, and Saturday, April 22 through 24, 1976.

Members of the Johns Hopkins faculty and guests will discuss basic mechanisms of intrauterine development and physiology. This year, special emphasis will be given to newer developments in fetal monitoring, including fetal respiratory activity, the oxytocin challenge test, and pulse rate variability, and to immunologic relationships between the maternal and fetal units. The visiting faculty includes Drs. E. J. Quilligan, Leon I. Mann, Alan E. Beer, Charles W. Hohler, and Harold E. Fox.

This year's conference will be held at the beautiful Hunt Valley Inn, Baltimore County, Maryland. Registration will be limited to the first 125 applicants. For additional information, contact: Dr. John W. C. Johnson, Department of Gynecology and Obstetrics, The Johns Hopkins Hospital, Baltimore, Maryland 21205.

Effects of fasting and theophylline on alanine-stimulated glucagon secretion in neonatal and infant sheep

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Plasma concentrations of glucose, alanine, and glucagon were measured after 24 hour fasting in newborn and infant sheep and in response to infusion of alanine alone and concurrently with theophylline. The plasma glucagon response to alanine was minimal in newborn sheep; in contrast, alanine produced a brisk response in plasma glucagon concentration in infant sheep. Glucose concentrations were unchanged in both groups. Theophylline enhanced blood glucagon and glucose responses to alanine in newborn animals but had minimal effects on the response of the infant sheep. These data, considered with earlier data in fetal sheep, suggest a progressive maturation of pancreatic islet alpha-cell glucagon secretion in the sheep during the postnatal period and suggest that the blunted glucagon response observed in the neonate is related to immaturity of the glucagon secretion mechanisms rather than deficient synthesis of the hormone. This immaturity may be related to impaired synthesis and/or enhanced degradation of cyclic adenosine monophosphate (cAMP) or to diminished responsiveness to cAMP.

IN PREVIOUS studies, using the in vivo fetal sheep model, we observed that the pancreatic islet alpha- and beta-cell secretion responses were immature at birth.[1, 2] Insulin secretion by the beta cell in response to hyperglycemia is minimal in utero[1] and remains obtunded at birth.[2] Glucagon secretion by the alpha cell in response to alanine is not observed in utero[1] and remains minimal in the newborn period.[2] Moreover, glucose does not suppress glucagon secretion in the newborn sheep as opposed to the adult.[2] The present studies were conducted in lambs beyond the newborn period to further characterize maturation of pancreatic islet alpha-cell glucagon secretion.

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Materials and methods

Thirteen healthy newborn sheep, weighing 4 to 5 kilograms and born spontaneously at term (146 to 150 days' gestation) to healthy date-bred ewes, and four 3-month-old (infant) sheep were used in the present studies. Jugular vein and carotid artery catheters were inserted and maintained patent with a dilute heparin solution (5 U per milliliter) until the time of study. Prior to study the heparin solution was withdrawn and normal saline was used as a flush during the study period. Newborn sheep were studied between 24 and 36 or 48 and 72 hours of age. They were separated from their mothers, restrained in an unanesthetized state, and studied after 24 hours of fasting; infant

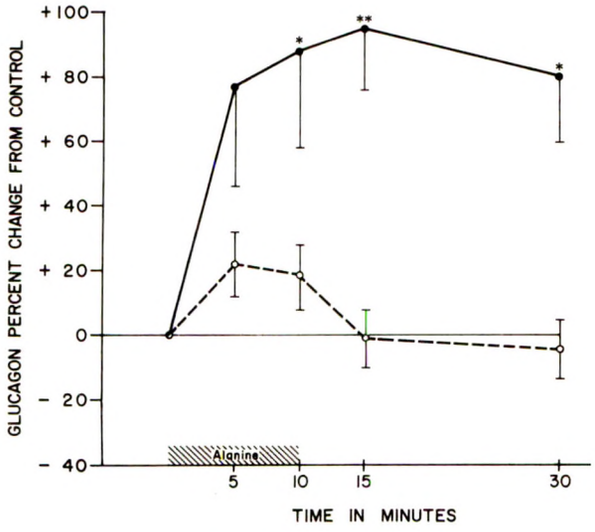


Fig. 1. Plasma glucagon concentrations plotted as per cent change from control in four infant sheep (closed circles, solid line) and 10 newborn sheep (open circles, dashed line) following the infusion of 1 mM. of alanine per kilogram of body weight. Values are plotted as mean and S.E.M. The single asterisk indicates that the values for the two groups of animals differ at the 0.05 level of significance. The double asterisks indicate differences significant at the 0.02 level.

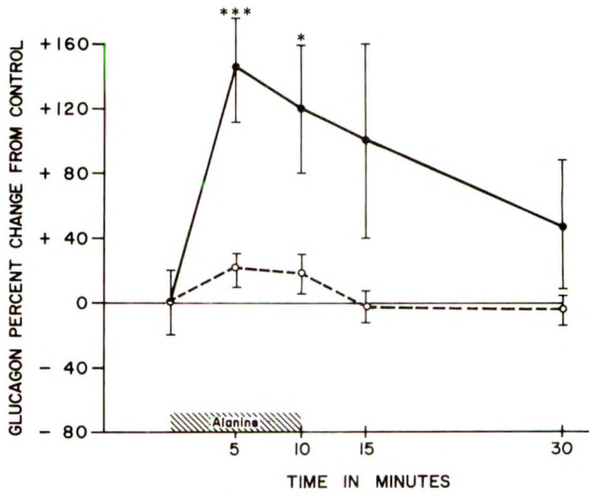


Fig. 2. Plasma glucagon concentrations plotted as per cent change from control in response to combined alanine-theophylline infusions in four newborn sheep (closed circles, solid line) and alanine-saline in 10 newborn sheep (open circles, dashed line). Values represent the mean and S.E.M. The single asterisk indicates that the values for the two groups of animals differ at the 0.05 level of significance. The triple asterisks indicate differences significant at the 0.001 level.

sheep also were studied after a 24 hour fast. In cases where the animals were used for more than one study, they were returned to the mother or were fed for 36 hours prior to another period of fasting. Each sheep received a 1 mM. per kilogram stimulation dose of L-alanine (Sigma Chemical Company) dissolved in 5 ml. of normal saline per kilogram and sterilized by filtering through a Millipore filter. One half the dose was given as a bolus injection over a 1 to 2 minute period and the remainder was infused over 10 minutes with a constant-infusion pump. In separate studies theophylline (5 mg. per kilogram) was administered concurrently with alanine in four newborn and four infant animals. Finally, two additional neonatal and two infant sheep were given theophylline alone 30 minutes prior to the alanine infusion.

Control blood specimens were obtained at three intervals over a 30 minute period during a saline infusion before the alanine or alanine plus theophylline infusions and at 5, 10, 15, 30, 45, and 60 minutes after beginning the infusions. Blood samples, (2.5 ml.) were collected in syringes rinsed in 10 per cent EDTA and promptly placed in chilled tubes containing EDTA plus 1,000 Kallikrein units of Traysylol. The blood remained iced until spun in a cold centrifuge at 2,000 r.p.m., after which the plasma was separated and stored at −4° C until assayed for glucagon, insulin, and glucose concentrations.

Glucose was measured by a glucose oxidase method,[3] alinine was measured by an alanine dehydrogenase method,[4] and glucagon was determined with a double-antibody radioimmunoassay developed in our laboratories.[5] The glucagon radioimmunoassay is sensitive to 20 pg. of crystalline glucagon per tube and does not cross-react significantly with gut glucagon. For statistical analysis, Student's t test was used to compare results in control and experimental animals.

Results

Basal plasma glucose concentrations averaged 68 ± 9 mg. per 100 ml. (mean and S.E.M.) in newborn and 59 ± 1 mg. per 100 ml. in infant animals after 24 hours of fasting. Glucose concentrations remained unchanged in both groups of animals after alanine infusion. Mean (and S.E.M.) basal glucagon concentrations were 101 ± 15 pg. per milliliter in newborn and 312 ± 71 pg. per milliliter in the infant sheep after fasting ($p < 0.02$). The mean blood glucagon concentration increased minimally in response to alanine in the neonates; the mean concentration observed at 5 minutes (134 ± 19 pg. per milliliter) was minimally but significantly ($p < 0.05$) higher than the mean fasting level by paired t test, but the increase was not sustained. The glucagon response to alanine was much more marked in the infant sheep with the mean level (624 ± 200) increased 78 per cent above the baseline

concentration by 10 minutes. Moreover, this increment was maintained throughout the 30 minute observation period.

These glucagon responses to alanine are graphically displayed in newborn and infant sheep in Fig. 1. For this comparison, since the mean baseline values were significantly different, the values are plotted at per cent change from baseline. The mean peak value in the infant animals was 214 ± 26 per cent over baseline vs. 132 ± 8 per cent in the newborn animals ($p < 0.001$).

Basal plasma alanine concentrations were 628 ± 25 mM. per liter after 24 hours' fasting in newborn sheep and 306 ± 24 mM. per liter after 24 hours' fasting in infant sheep ($p < 0.001$).

To assess the possible role of AMP in the mediation of glucagon secretion, theophylline, a phosphodiesterase inhibitor, was infused concurrently with alanine; the results are depicted in Fig. 2. In the four newborn sheep theophylline infusion produced a marked augmentation of alanine-stimulated glucagon secretion (from a mean baseline value of 117 ± 10 to 245 ± 32 pg. per milliliter maximum response, $p < 0.001$). In contrast, although theophylline tended to augment the glucagon stimulation response in the 3-month-old sheep, the mean maximal increase was not statistically significant. The increase in plasma glucose concentrations following alanine infusion also tended to be greater with theophylline augmentation in the newborn as well as the infant sheep. The mean maximal increase approximated 20 per cent above basal levels in both groups ($p < 0.05$).

In the two newborn and two older sheep infused with theophylline alone, no change in glucose or glucagon concentrations was seen over the 30 minute observation period. As an additional control, a 10 mM. per kilogram dose of alanine (rather than 1 mM. per kilogram) was infused into two newborn sheep fasted 24 hours. The glucagon response to the larger dose of alanine was similar to the response to the 1 mM. per kilogram dose. There were no correlations among the glucagon, glucose, or alanine responses in either the newborn or infant animals.

Comment

The present studies have shown that plasma glucagon levels increase minimally in the newborn sheep in response to prolonged fasting or to alanine infusion. Infant sheep had higher mean basal blood glucagon levels after a 24 hour fast and alanine stimulation produced a rapid and marked further increase in mean glucagon concentration. These responses indicated that significant maturation of the pancreatic islet alpha-cell secretion response occurs during the early weeks of postnatal life in the sheep. In the human newborn infant glucagon and glucose concentrations in cord blood also have been reported to increase in response to alanine infused into the mother during labor.[6]

This rise in glucagon in response to alanine infused in the mother could have resulted from other factors. We have reported a paradoxical glucagon response to glucose in the newborn sheep.[2] Thus an increase in transplacental glucose might have been involved, and it is possible that this increase was not due to the alanine. Also, it has been reported that plasma glucagon concentrations are not suppressed in response to increased blood glucose levels produced during exchange transfusion.[7, 8] Finally, we have shown that the human newborn infants increase plasma glucagon levels only slightly in response to oral[9] or intravenous alanine.[5] The present studies in sheep and these quoted studies in the human neonate suggest that the mechanism(s) for control of glucagon secretion is immature at birth. It is interesting that the newborn sheep sustained his glucose so well with fasting compared to the infant, suggesting that gluconeogenesis proceeds adequately even at low basal glucagon levels.

It has been reported recently that theophylline enhances glucagon secretion in pancreatic islet cell preparations studied in vitro, suggesting a relationship between the cAMP generating system and glucagon secretion.[10] In addition, Chez, Mintz, and Hutchinson[11] have reported an enhanced insulin response in the subhuman primate when theophylline is infused concurrently with glucose. The enhanced glucagon response to alanine produced by theophylline in the present studies suggests that glucagon synthesis is not limiting secretion. The blunted glucagon response in the neonatal sheep and the augmented response observed during theophylline infusion could result either because cAMP generation in response to stimulation is relatively decreased in the neonate and/or baseline phosphodiesterase activity is relatively increased.

The plasma concentrations of alanine might influence glucagon secretion and increased alanine levels have been thought to augment glucagon secretion[12]; however, the high basal levels could dampen glucagon secretion in response to alanine in the neonate. Even if this were so, the enhanced glucagon response with theophylline indicates that such a depressive effect is overcome with a phosphodiesterase inhibitors.

The present studies confirm that the mechanism(s) for glucagon secretion, like that for insulin secretion, in

fetal and newborn animals is immature at birth and that secretion in response to alanine infusion can be augmented by theophylline. The slow maturation of this mechanism(s) during the fetal life may be related to slow maturation of the cAMP-generating system.

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The dosage regimen with LO/OVRAL is convenient and easy to follow: 3 weeks on, 1 week off. The attractive, uncomplicated Pilpak® dispenser helps patients follow the right dosage schedule.

*Serious as well as minor adverse reactions have been reported following the use of all oral contraceptives. The physician should remain alert to their earliest manifestations and in the event of serious adverse reactions should avoid any oral contraceptives. *Special note, contraindications, warnings, precautions and adverse reactions* in the accompanying IN BRIEF should be carefully considered.

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IN BRIEF:

SPECIAL NOTE: Oral contraceptives have been marketed in the United States since 1960. Reported pregnancy rates vary from product to product. The effectiveness of the sequential products appears to be somewhat lower than that of the combination products. Both types provide almost completely effective contraception.

An increased risk of thrombotic, thromboembolic and cerebral-vascular disease associated with the use of hormonal contraceptives has been shown in studies conducted in both Great Britain and the United States. Other risks, such as those of elevated blood pressure and reduced tolerance to carbohydrates, have been reported, and appropriate tests should be conducted to monitor these during oral-contraceptive therapy. Liver disease has also been reported, and the physician should be alert to its earliest manifestations.

Long-term administration of both natural and synthetic estrogens in subprimate animal species in multiples of the human dose increases the frequency for some animal carcinomas. These data cannot be transposed directly to man. The possible carcinogenicity due to the estrogens can neither be affirmed nor refuted at this time. Close clinical surveillance of all women taking oral contraceptives must be continued.

INDICATIONS: For oral contraception.

CONTRAINDICATIONS: 1. Thrombophlebitis, thromboembolic disorders, cerebral-vascular disease, or a past history of these conditions; 2. Markedly impaired liver function; 3. Known or suspected carcinoma of the breast; 4. Known or suspected estrogen-dependent neoplasia; 5. Undiagnosed abnormal genital bleeding; 6. Known or suspected pregnancy.

WARNINGS: 1. The physician should be alert to the earliest manifestations of thrombotic and thromboembolic disorders, thrombophlebitis, cerebrovascular disorders including hemorrhage, pulmonary embolism and retinal thrombosis. Should any of these occur or be suspected, the drug should be discontinued immediately.

Retrospective studies of morbidity and mortality in Great Britain and studies of morbidity in the United States have shown a statistically significant association between thrombophlebitis, pulmonary embolism and cerebral-vascular disease, occlusive or hemorrhagic, and the use of oral contraceptives. There have been three principal studies in Great Britain[1-3] leading to these conclusions and three[4-7] in this country. The estimate of the relative risk of thromboembolism in the study by Vessey and Doll[3] was about sevenfold, while the United States studies found relative risks of 4.4 to 11, meaning that the users are several times as likely to undergo thromboembolic disease without evident cause as non-users.

In May, 1974, the Royal College of General Practitioners in England[8] issued an interim report of its continuing large-scale prospective study comparing a user group to a non-user group. This study in its interim analysis states: "A statistically significant higher rate of reporting of cerebrovascular accidents in Takers is evident, but the numbers are too small to justify an estimation of the degree of risk." The study also reported a higher incidence of superficial and deep vein thrombosis in users as compared to non-users.

The Sartwell study[4] indicated that the risk did not persist after discontinuation of administration. Both the Sartwell and the Royal College study indicated that the degree of risk was not associated with duration of treatment.

In a collaborative American study[5,6] of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the relative risk of thrombotic stroke was 4.1 to 9.5 times greater in users than in non-users. A comparable estimate for hemorrhagic stroke was 2.0.

None of the American studies was designed to evaluate a difference between products. However, the Sartwell study[4] suggested that there might be an increased risk of thromboembolic disease in users of sequential products.

Other retrospective studies[9,10] have reported an increased risk of post-surgery thromboembolic complications in oral-contraceptive users. It has been recommended that therapy be discontinued at least one month prior to elective surgery.

2. Discontinue oral-contraceptive medication if there is: gradual or sudden, partial or complete loss of vision; proptosis or diplopia; onset or aggravation of migraine or development of headache of a new pattern which is recurrent, persistent or severe; papilledema; or any evidence of retinal-vascular lesions. 3. Fetal abnormalities have been reported to occur in the offspring of women who have taken progestogens and/or estrogens during pregnancy.[11,12] The safety of LO/OVRAL® in pregnancy has not been demonstrated. Pregnancy should be ruled out before initiating or continuing the contraceptive regimen. Pregnancy should always be considered if withdrawal bleeding does not occur. 4. A small fraction of the hormonal agent in oral contraceptives has been identified in the milk of mothers receiving these drugs. The long-range effect to the nursing infant cannot be determined at this time. 5. Hepatic lesions (adenomas, hepatomas, hamartomas, regenerating nodules, etc.), occasionally fatal, have been reported in women on oral contraceptives. Such lesions may present as an abdominal mass or with the signs and symptoms of an acute abdomen. These lesions should be considered if the patient has abdominal pain or evidence of intra-abdominal bleeding. Such lesions have been reported in short-term as well as long-term users of oral contraceptives.

PRECAUTIONS: 1. A thorough history and physical examination should be performed before prescribing oral contraceptives and periodically during their administration and should include special reference to breasts and pelvic organs including Papanicolaou smears, since estrogens have been known to produce tumors, some of them malignant, in five species of subprimate animals. 2. Endocrine and possibly liver-function tests may be affected by treatment with LO/OVRAL® Therefore, if such tests are abnormal in a patient taking LO/OVRAL® it is recommended that they be repeated after the drug has been withdrawn for two months. 3. Under the influence of estrogen-progestogen preparations, pre-existing uterine fibromyomata may increase in size. 4. Because these agents may cause some degree of fluid retention, conditions which might be influenced by this factor, such as epilepsy, migraine, asthma, cardiac or renal dysfunction, require careful observation. 5. In breakthrough bleeding, and in all cases of irregular bleeding per vaginam, nonfunctional causes should be borne in mind. In undiagnosed bleeding per vaginam, adequate diagnostic measures are indicated. 6. Patients with a history of psychic depression should be carefully observed and the drug discontinued if the depression recurs to a serious degree. 7. Any possible influence of prolonged LO/OVRAL® therapy on pituitary, ovarian, adrenal, hepatic or uterine function awaits further study. 8. A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. The mechanism of this decrease is obscure. For this reason, diabetic patients should be carefully observed while receiving LO/OVRAL® therapy. 9. The age of the patient constitutes no absolute limiting factor, although treatment with LO/OVRAL® may mask the onset of the climacteric. 10. The pathologist should be advised of LO/OVRAL® therapy when relevant specimens are submitted. 11. Susceptible women may experience an increase in blood pressure following administration of contraceptive steroids. 12. Cholestatic jaundice has been reported in users of oral contraceptives. If this occurs, LO/OVRAL® should be discontinued. This condition is more likely to occur in patients who have experienced cholestatic jaundice of pregnancy. Patients with a history of cholestatic jaundice of pregnancy should be carefully observed during LO/OVRAL® therapy.

ADVERSE REACTIONS OBSERVED IN PATIENTS RECEIVING ORAL CONTRACEPTIVES: A statistically significant association has been demonstrated between use of oral contraceptives and the following serious adverse reactions: thrombophlebitis, pulmonary embolism, cerebral thrombosis and hemorrhage, gall bladder disease.

Although available evidence is suggestive of an association, such a relationship has been neither confirmed nor refuted for the following serious adverse reactions: neuro-ocular lesions, e.g., retinal thrombosis, optic neuritis and hepatic lesions with or without intra-abdominal bleeding.

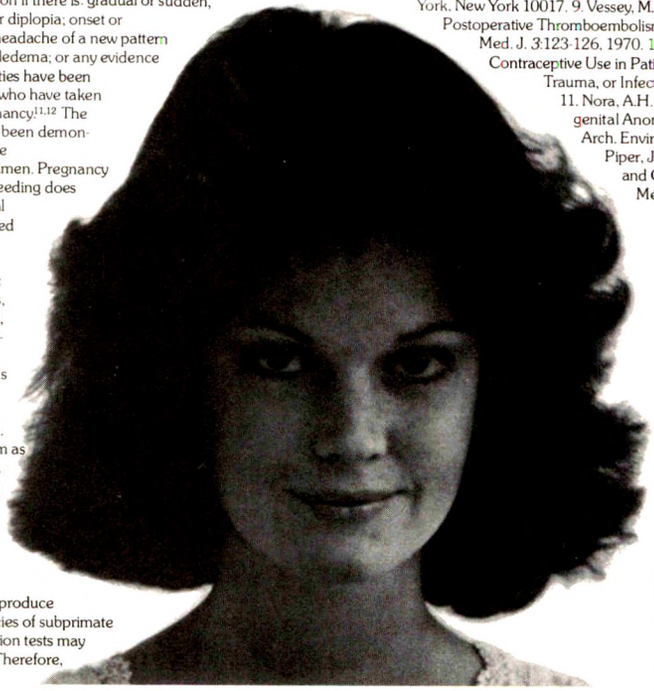
The following adverse reactions are known to occur in patients receiving oral contraceptives: nausea, vomiting, gastrointestinal symptoms (such as abdominal cramps and bloating), breakthrough bleeding, spotting, change in menstrual flow, amenorrhea during and after treatment, edema, chloasma or melasma, which may persist, breast changes: tenderness, enlargement and secretion, change in weight (increase or decrease), changes in cervical erosion and cervical secretions, suppression of lactation when given immediately post-partum, cholestatic jaundice, migraine, rash (allergic), rise in blood pressure in susceptible individuals, mental depression.

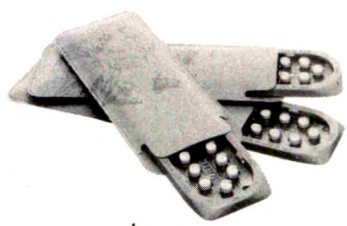
Although the following adverse reactions have been reported in users of oral contraceptives, an association has been neither confirmed nor refuted: anovulation post treatment, which tends to occur more frequently in women with a history of menstrual disorders, premenstrual-like syndrome, changes in libido, changes in appetite, cystitis-like syndrome, headache, intolerance to contact lenses, nervousness, dizziness, fatigue, backache, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, itching and vaginitis.

The following laboratory results may be altered by the use of oral contraceptives: hepatic function; increased sulfobromophthalein retention and other tests; coagulation tests; increase in prothrombin, Factors VII, VIII, IX and X; decrease in antithrombin III; increase in platelet aggregability; thyroid function: increase in PBI, and butanol extractable protein-bound iodine and decrease in T^3 uptake values; metyrapone test; and pregnanediol determination.

NOTE: For more specific details on LO/OVRAL, refer to package insert.

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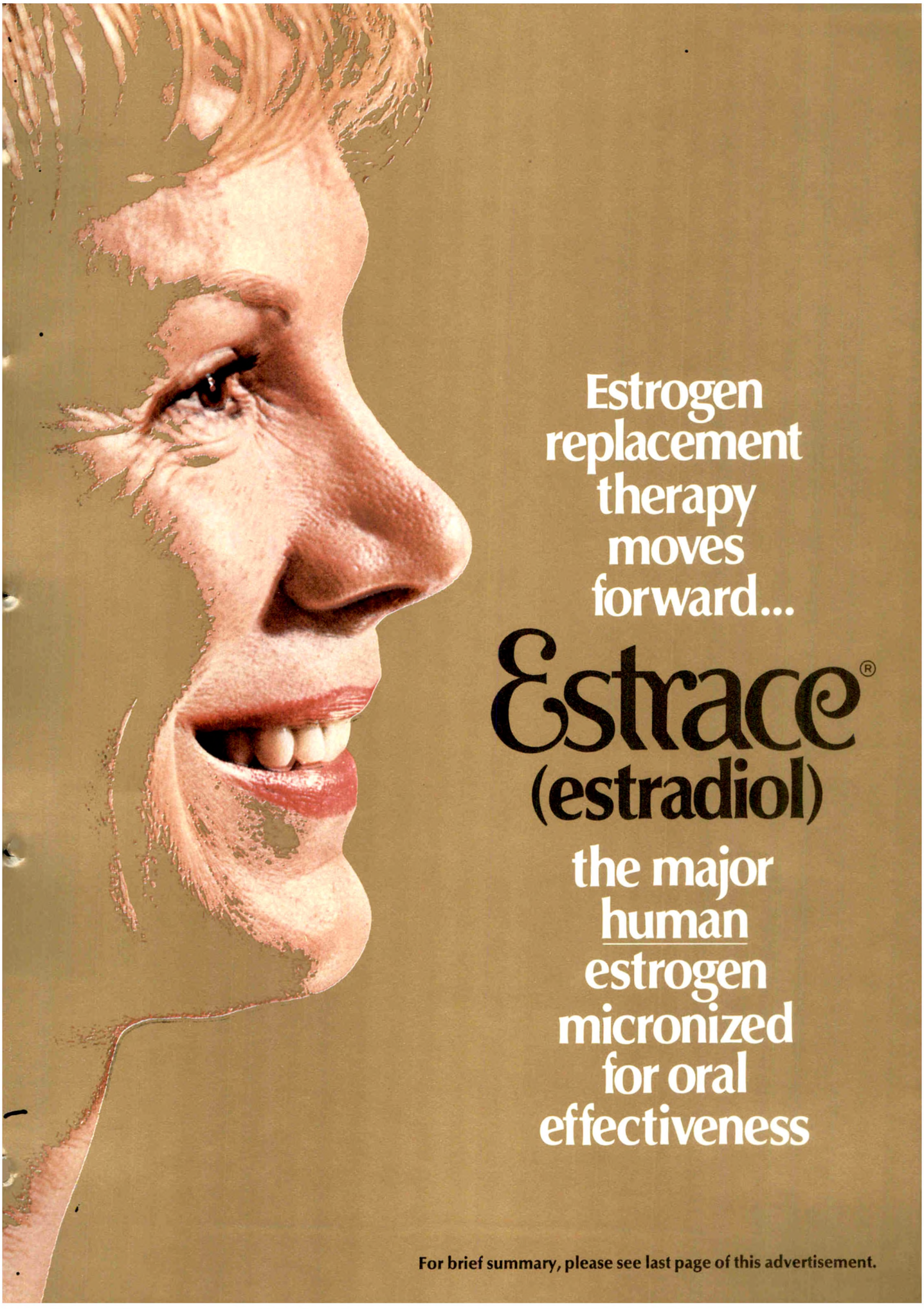




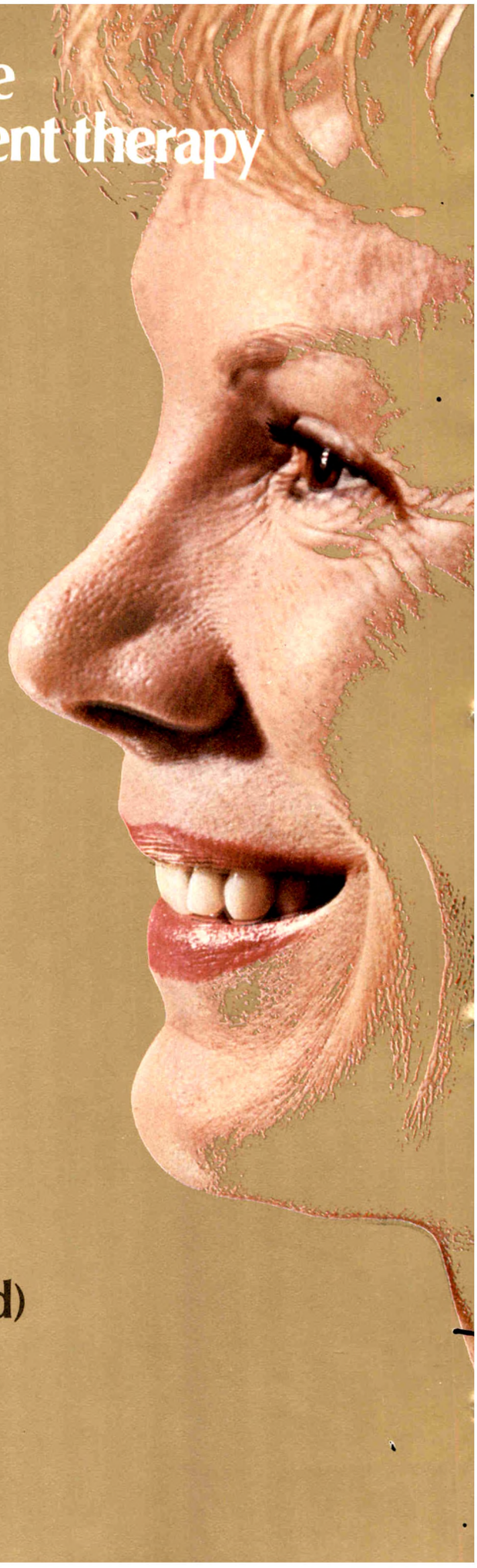
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- synthesized in free form (not esterified, not conjugated)

overall, 95% of patients studied gained adequate or complete relief of their symptoms[1]

Estrace (estradiol) was given to 369 estrogen-deficient women for periods ranging up to a year or more (average of six months); 319 of these women received an initial dosage of 1 or 2 mg. per day and were evaluated for efficacy. *Complete disappearance* of hot flushes was experienced by 227 (76%) of 298 women, of sweating by 131 (80%) of 164 women, of tingling by 62 (94%) of 66 women, and of genital atrophy by 44 (88%) of 50 women. In general, 95% of the patients evaluated experienced either adequate or complete relief of their symptoms. Remarkably, in 77% of the patients, symptoms were controlled without need to adjust the initial dosage—usually 2 mg. daily. (Dosage after adjustment ranged from 1 mg. to 3 or 4 mg. a day.)[1]

estradiol frequently desired

Physicians for years have desired to use estradiol, the major human ovarian hormone, as the basis for oral estrogen replacement therapy. Since 1953, several studies have reported that oral administration of estradiol, in 1 to 2 mg. doses, provided varying degrees of relief of menopausal symptoms.[2–4] One study[4] showed that the optimum estrogenic effect was obtained with 2 mg. daily.

In a preliminary investigation, Martin, *et al.*,[5] 1972, reported on a single-blind study of 71 women given four different estrogenic preparations. A formulation of micronized estradiol was selected by more women than any other preparation. In fact, twice as many selected micronized estradiol than conjugated equine estrogen.* Yen, *et al.*,[6] showed that micronized estradiol (Estrace®) given orally is readily absorbed, metabolized in part to estrone, and is biologically active.

With micronized estradiol there is no need for special coatings which may hamper tablet absorption and no "barnyard" aftertaste—problems sometimes seen with conjugated equine estrogens.

*Because the micronized estradiol formulations may differ, these data are not necessarily applicable to Estrace.

estradiol...orally effective

The technique of micronization has opened up the oral route to natural human estrogen replacement with Estrace. Reduced particle size of the estradiol in Estrace makes effective gastrointestinal absorption possible. Onset of action is rapid, with marked increases in levels of circulating estradiol and estrone; concomitant decreases in serum concentrations of FSH and LH are sustained over a 24-hour period.[6]

Estrace apparently creates a reservoir of physiologically active estrogen that is available to the cells on an "as needed" basis.[6] And because estradiol can be assayed in plasma, Estrace can provide objective[6] as well as clinical evidence[1] of ready availability and biologic activity.

well accepted by patients[1]

In the 369 patients previously described, the incidence of estrogen-induced side effects with Estrace was relatively low. Incidence of nausea was remarkably low—only 2% of patients before dosage adjustment. Some patients who had complained of this problem while on conjugated equine estrogens reported its complete disappearance on changing to Estrace. Whereas before dosage adjustment 24% experienced breast tenderness, 31% edema, 18% (of 151 with intact uteri) bleeding, and 4% weight gain, only about 2% of the 369 patients dropped out because of side effects.[1]

A few instances of significant gynecologic disease became apparent during clinical investigations.[1] Endometrial hyperplasia was discovered in seven patients, all of whom had previously received estrogen therapy for one to three years prior to entering the study. One patient was reported to have carcinoma *in situ* and another showed adenocarcinoma of the endometrium. In clinical practice, the physician should be alerted to the potential of thrombotic disorders and be prepared to discontinue medication immediately if they occur. Because of variations in normal endogenous hormone production, certain patients may exhibit signs of excessive estrogenic stimulation. Adverse reactions generally associated with estrogen administration may occur and these are listed in the brief summary. Prior to initiation of treatment with Estrace or any other estrogenic preparation, an adequate physical examination should be given.

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For Brief Summary, please see following page.

Estrogen replacement therapy moves forward...



Estrace® (estradiol)

the major human estrogen micronized for oral effectiveness

Indications: Estrace® (17β-Estradiol) is indicated for relief of endogenous estrogen deficiency and associated menopausal symptoms.

Contraindications: Estrace® is contraindicated in patients with 1) markedly impaired liver function; 2) known or suspected carcinoma of the breast, except those cases of progressing disease not amenable to surgery or irradiation occurring in women who are at least 5 years postmenopausal, or in men in whom castration is not feasible; 3) known or suspected estrogen dependent neoplasia, such as carcinoma of the endometrium; 4) undiagnosed abnormal genital bleeding; 5) thrombophlebitis, thromboembolic disorders, cerebral apoplexy, or with a past history of these conditions; and 6) pregnancy.

Warnings: The physician should be alert to the earliest manifestations of thrombotic disorders (thrombophlebitis, cerebrovascular disorders, pulmonary embolism and retinal thrombosis). If these occur or are suspected the drugs should be discontinued immediately. Discontinue medication pending examination if there is a sudden onset of proptosis, diplopia, or migraine. If examination reveals papilledema or retinal vascular lesions, medication should be withdrawn. Estrogens may be excreted in the mother's milk and an estrogenic effect upon the infant has been described. The long range effect on the nursing infant cannot be determined at this time. Hypercalcemia may occur in as many as 15% of breast cancer patients with metastases and this usually indicates progression of bone metastases. This occurrence depends neither on dose nor on immobilization. In the presence of untoward effects such as progression of the cancer or hypercalcemia, estrogen medication should be stopped. A statistically significant association has been reported between maternal ingestion during pregnancy of diethylstilbestrol and the occurrence of vaginal carcinoma developing years later in the offspring. Whether such an association is applicable to all estrogens is not known at this time. In any event, estrogens are not indicated for use during pregnancy.

Precautions: Because normal endogenous hormone production varies individually, certain patients may be unusually responsive to estrogenic therapy and may respond with undesirable manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding, mastodynia, edema, etc. Because of estrogen induced salt and water retention, these drugs should be used with caution in patients with epilepsy, migraine, asthma, cardiac or renal disease. Patients with a history of psychic depression should be carefully observed and the drug discontinued if the depression recurs to a serious degree. In the event that any unexplained or excessive vaginal bleeding would occur while on estrogen therapy, nonfunctional causes should be borne in mind. The drug should be discontinued and a thorough investigation made as to the cause, being certain to rule out the possibility of malignancy. Pre-existing uterine fibromyomata may increase in size while using this product, therefore, patients should be examined at regular intervals while receiving estrogenic therapy. Women with a strong family history of cancer, recurrent chronic cystic mastitis, or abnormal mammograms should be administered estrogens with caution. Because of a possible decrease in glucose tolerance, diabetic patients should be followed closely. Because estrogens influence the metabolism of calcium and phosphorus, they should be used with caution in patients with certain metabolic bone diseases that are associated with hypercalcemia or in patients with renal insufficiency. The pathologist should be advised of estrogen therapy when relevant specimens are submitted. Because of the effects of estrogens on epiphyseal closure, they should be used judiciously in young patients in whom bone growth is not complete. A pre-treatment physical examination should include special reference to breast and pelvic organs as well as a Papanicolaou smear since estrogens have been known to produce tumors, some of them malignant, in five species of animals. When large doses of estrogens are used, urinary stress incontinence may occur in nonpregnant females. Prolonged high doses of estrogens will inhibit anterior pituitary functions. This should be borne in mind when treating patients in whom fertility is desired. Continuous use of estrogens will result in prolonged stimulation of the endometrium and breast. In order to avoid this, oral estrogens should be administered cyclically in the menopausal or hypogonadal patient. The age of the patient constitutes no absolute limiting factor, although treatment with estrogens may mask the onset of the climacteric. Certain endocrine and liver function tests may be affected by treatment with estrogens. If such tests are abnormal in a patient taking these drugs it is recommended that they be repeated after the drug has been withdrawn for two months. Any possible influence of prolonged estrogen therapy on pituitary, ovarian, adrenal, hepatic, or uterine function awaits further study.

Adverse Reactions: A statistically significant association has been demonstrated between use of estrogen-progestin oral contraceptives and the following serious reactions: Thrombophlebitis, pulmonary embolism, and cerebral thrombosis. Although available evidence is suggestive of an association, such a relationship has been neither confirmed nor refuted for the following serious reactions: coronary thrombosis and neuro-ocular lesions (e.g. retinal thrombosis and optic neuritis). The following adverse reactions are known to occur in patients receiving estrogens: nausea, vomiting, anorexia, gastrointestinal symptoms (such as abdominal cramps or bloating), edema, breakthrough bleeding, spotting or withdrawal bleeding, breast tenderness and enlargement, change in body weight (increase or decrease), headache, increased cervical mucus, allergic rash, loss of libido and gynecomastia in the male, reactivation of endometriosis, aggravation of migraine headaches, hepatic cutaneous porphyria becoming manifest, cholestatic jaundice, rise in blood pressure in susceptible individuals, mental depression, cystitis-like syndrome, loss of scalp hair, erythema nodosum, hemorrhagic eruption, premenstrual-like syndrome, changes in libido, changes in appetite, nervousness, dizziness, fatigue, backache, erythema multiforme, itching, possible diminution in lactation when given immediately post-partum, irritability, and malaise.

Important Notes: An increased risk of thromboembolic disease associated with the use of oral contraceptives containing estrogens and progestins has now been conclusively established. Retrospective studies have shown a statistically significant association between thrombophlebitis, pulmonary embolism, and cerebral thrombosis and embolism and the use of these drugs. There have been three principal studies in Great Britain[1-3] and one in the United States[4] leading to this conclusion. As a result of these studies, it has been estimated that users of oral contraceptives containing estrogens are 4 to 7 times more likely than nonusers to develop thromboembolic disease without evident cause. The American study also indicated that the increased risk did not persist after discontinuation, nor was it enhanced by long continued administration. Although the American study was not designed to evaluate a difference between products, it did suggest that there might be an increased risk of thromboembolic disease in users of sequential products. Confirmation of this finding requires further study. In a more recent analysis of data derived from several national adverse reaction reporting systems,[5] British investigators concluded that the risk of thromboembolism, including coronary thrombosis, is directly related to the dose of estrogen used in oral contraceptive products. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. Nevertheless, in view of this study, as well as others that have demonstrated a positive relationship between estrogens and thromboembolism, it would seem prudent and in keeping with basic therapeutic principles, to utilize, whenever feasible, the smallest effective dose of estrogen in treating patients. Risks associated with certain other known adverse reactions, such as elevated blood pressure, liver dysfunction, and reduced tolerance to carbohydrate, have not as yet been quantitated. Long term administration of both natural and synthetic estrogens in subprimate animal species in multiples of the human dose increases the frequency for some animal carcinomas. These data cannot be transposed directly to man. The possible carcinogenicity due to the estrogens can neither be confirmed nor refuted at this time. Close clinical surveillance of all women taking estrogens must be continued. It has been reported in a recent study done in the United States[6] that the maternal ingestion of diethylstilbestrol during pregnancy appears to increase the risk of vaginal adenocarcinoma developing years later in the offspring exposed.

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Dosage and Administration: Treatment is usually initiated with a dose of 1 or 2 mg. daily of micronized estradiol. Dosage may be adjusted to control the presenting symptoms depending on the response of the individual patient; the minimal effective dose for maintenance therapy should be determined by titration. To avoid continuous stimulation of the reproductive tract and breasts, cyclic therapy is recommended. The usual regimen consists of three weeks on drug followed by one week off drug. Some patients may require a slightly shorter estrogen-free interval (4-6 days) to prevent recurrence of menopausal symptoms. Withdrawal bleeding of unusual amount or duration should be investigated.

How Supplied: Estrace® 1 mg; lavender scored tablets, NDC 0087-0755-01, bottles of 100. Estrace® 2 mg; turquoise scored tablets, NDC 0087-0756-01, bottles of 100.

Mead Johnson LABORATORIES

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While every precaution is taken to insure accuracy, we cannot guarantee against the possibility of an occasional change or omission in the preparation of this index.

Non-Invasive Fetal Monitoring: Fact vs. Fiction.

For more than fifteen years there has been little argument that the fetal ECG – the most accurate and reliable basis from which to measure the fetal heart rate – was available only through invasive, scalp electrode techniques.

That is no longer true.



t: The new Brattle Fetal Monitor from B-D Electrodyne provides continuous, accurate FHR tracings derived from the abdominal fetal ECG.

Until recently, attempts to isolate the fetal ECG from the combined ECG signal taken from the maternal abdomen have failed. So complex was the problem that the abdominal fetal ECG method had been largely abandoned in favor of ultrasound and phonocardiography. Present non-invasive instrumentation was designed around these older methods.

Technology was not available to satisfactorily solve 3 important problems associated with abdominal fetal ECG.

1. The maternal ECG signal, often five times as large as that of the fetal ECG, would dominate.
2. The fetal ECG was often masked by the electrical signals (noise) produced by the abdominal muscles, especially during contractions.
3. The fetal ECG complex was difficult to distinguish from the maternal ECG complex when the two occurred simultaneously.

Those are no longer problems.

Fact: The Brattle Fetal Monitor separates the fetal ECG from the maternal ECG, and presents the fetal heart rate in beat-to-beat fashion.

Through patented circuitry, the fetal ECG signal is amplified and the larger maternal ECG is eliminated. The obstinate problem of removing electrical signals produced during most contractions is overcome by electronic filtering. Finally, the Brattle Fetal Monitor continues to discriminate between the fetal QRS complex and the maternal QRS complex, even when the two are merged. In addition, the monitor can be used with a scalp electrode or with an optional ultrasound amplifier. Uterine activity is simultaneously recorded on the second channel.



Top Strip Chart: FHR tracing from scalp electrode.
Bottom Strip Chart: FHR tracing from Brattle Fetal Monitor using abdominal electrodes.

Sample FHR tracings above were obtained by simultaneous monitoring and are virtually identical.

B-D and Electrodyne are trademarks of Becton, Dickinson and Company.

Fact: The Brattle Fetal Monitor has been clinically evaluated for more than 26 months.

Through nearly 2500 hours of labor with more than 500 patients, during which there were numerous comparisons with simultaneously recorded tracings taken from fetal-attached electrodes, the "Brattle" showed accuracy and reliability remarkably close to the performance of the directly attached electrodes and significantly better than ultrasonic methods.

Fact: The Brattle Fetal Monitor is so accurate and reliable that the ultrasound method is offered only as an option.

Fact: The Brattle Fetal Monitor offers significant diagnostic and operational advantages.

1. The important difference between abdominal fetal ECG and the ultrasound method is in the quality and reliability of our tracings. Whereas the ultrasound technique interprets the FHR indirectly from movements of the fetal heart, B-D Electrodyne's new monitor derives the FHR directly from the fetal ECG.
2. Applying the monitor is quick and simple. Placement of abdominal electrodes does not require a search procedure, and there is no need to reposition electrodes as labor progresses. Only two electrodes are required.
3. Switch-over to direct, fetal-attached scalp electrode is quickly and easily accomplished, if desired.
4. An audible tone representing each maternal heart beat can be obtained by pressing a front panel switch.
5. Labor is easily recorded with a lightweight tocodynamometer.
6. The Brattle Fetal Monitor has a trend recorder, which can be switched to the faster speed of 25 mm/sec for recording of both fetal and maternal ECG's.

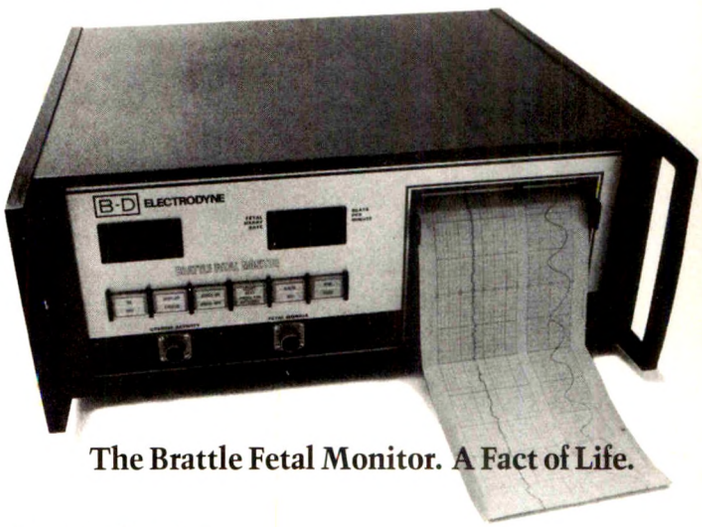
Fact: The Brattle Fetal Monitor from B-D Electrodyne is a complete system.

Digital display of fetal heart rate.
Non-fade scope display of ECG (optional).
Selection of disposable abdominal electodes, or disposable scalp electrodes, or ultrasound transducer (optional), and labor transducer.
Trend and fast speed recorder for FHR, labor and ECG.
Safe for the operator, the mother, and the baby.

Fact: Twenty-five years of experience.

Now you know about B-D Electrodyne's involvement in fetal monitoring. But perhaps you didn't know that we were one of the very first into this business of electronically monitoring the human heart twenty-five years ago. We are as confident today of the medical importance of abdominal fetal ECG as we were of adult heart monitoring way back then.

In addition, we're backed by our parent company, Becton, Dickinson and Company, number one in hospital products for over seventy-five years.



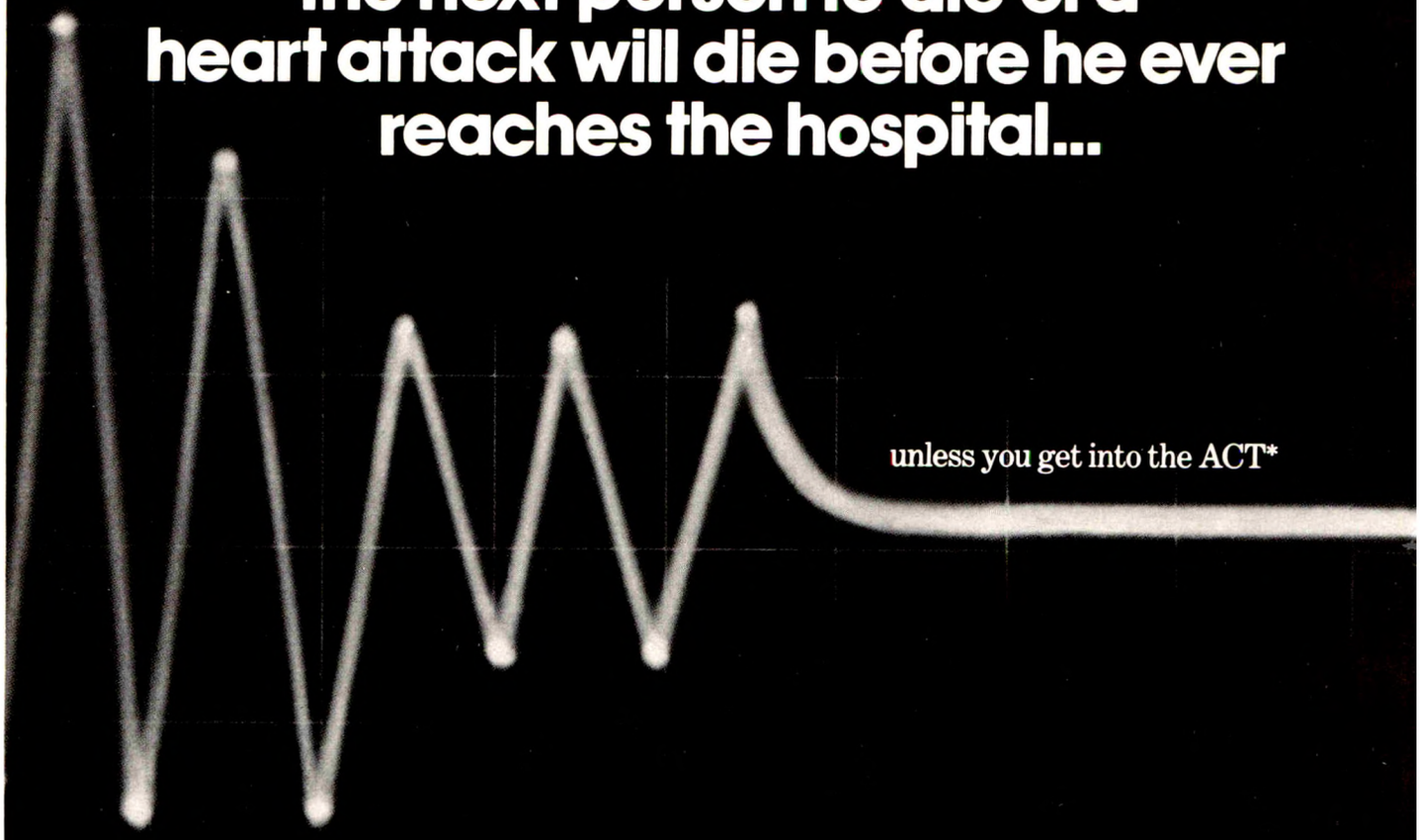
The Brattle Fetal Monitor. A Fact of Life.

Fact vs. Fiction

Some may continue to claim that reliable abdominal fetal ECG is still fiction. We're ready to prove to you in your own facility that abdominal fetal ECG is now a fact. To arrange for a demonstration of the Brattle Fetal Monitor please write to us at B-D Electrodyne, Division of Becton, Dickinson and Company, Route One, Sharon, Massachusetts 02067, or call us at 617-828-9080.

B-D **ELECTRODYNE**
Division of Becton, Dickinson and Company

Chances are 50/50 the next person to die of a heart attack will die before he ever reaches the hospital...



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D-14084-4

CURRENT INVESTIGATION

This section offers prompt first announcement of new observations or discoveries. Articles should be limited to 1,500 words and 6 references. Illustrations or additional references require a proportionate reduction in total words.

Luteolytic and abortifacient effects of serial intramuscular injections of 15(S)-15-methyl-prostaglandin $F_{2\alpha}$ in early pregnancy

NIELS H. LAUERSEN, M.D.
KATHLEEN H. WILSON, B.S.
New York, New York

Pregnancy was successfully terminated in 8 of 9 patients 5 to 6 weeks after the last menstrual period by serial intramuscular injections of 15(S)-15-methyl-prostaglandin $F_{2\alpha}$ (15-ME-$PGF_{2\alpha}$). In one patient the treatment induced vaginal bleeding and a drop in hormone levels, but the patient did not completely abort and the uterus had to be evacuated. Serial intramuscular injections of 15-ME-$PGF_{2\alpha}$ appeared to have some luteolytic effect, resulting in a significant drop in progesterone and estradiol levels. However, it is uncertain if the luteolytic effect of the prostaglandin was the precipitating factor in the abortion since the administration of 15-ME-$PFG_{2\alpha}$ was accompanied by strong uterine activity which played a role in the abortion.

PROSTAGLANDIN $F_{2\alpha}$ ($PGF_{2\alpha}$) has been shown to have a luteolytic effect in laboratory animals, but no consistent evidence is available for a luteolytic effect of prostaglandin in the human subject. Wentz and Jones[1] studied the effect of $PGF_{2\alpha}$ infusion in 13 women with delayed menses. Four nonpregnant patients experienced normal menstrual bleeding, and 5 of the 8 pregnant patients aborted after 8 hours of intravenous $PGF_{2\alpha}$ infusion at a rate of 50 μg per minute.

Powell and associates[2] demonstrated the presence of $PGF_{2\alpha}$ receptor in the human corpus luteum and suggested that prostaglandin has a physiologic role in human luteolysis. These authors suggested that $PGF_{2\alpha}$ is synthesized locally by the ovary, rendering the human corpus luteum less sensitive to exogenous $PGF_{2\alpha}$. A luteolytic effect might be shown by prolonged administration of high doses of prostaglandin, but unfortunately administration of the naturally occurring $PGF_{2\alpha}$ in doses that may have a luteolytic effect is not feasible due to the occurrence of severe gastrointestinal side effects. Prostaglandin analogues have been demonstrated to have increased potency and abortifacient effectiveness by the intramuscular route.[3]

From the Department of Obstetrics and Gynecology, The New York Hospital–Cornell Medical Center.

Supported by Grant No. 73049 from The Rockefeller Foundation, and by The Upjohn Co., 7171 Portage Rd., Kalamazoo, Michigan 49001.

Reprint requests: Dr. Niels H. Lauersen, Department of Obstetrics and Gynecology, The New York Hospital–Cornell Medical Center, 525 E. 68th St., New York, New York 10021.

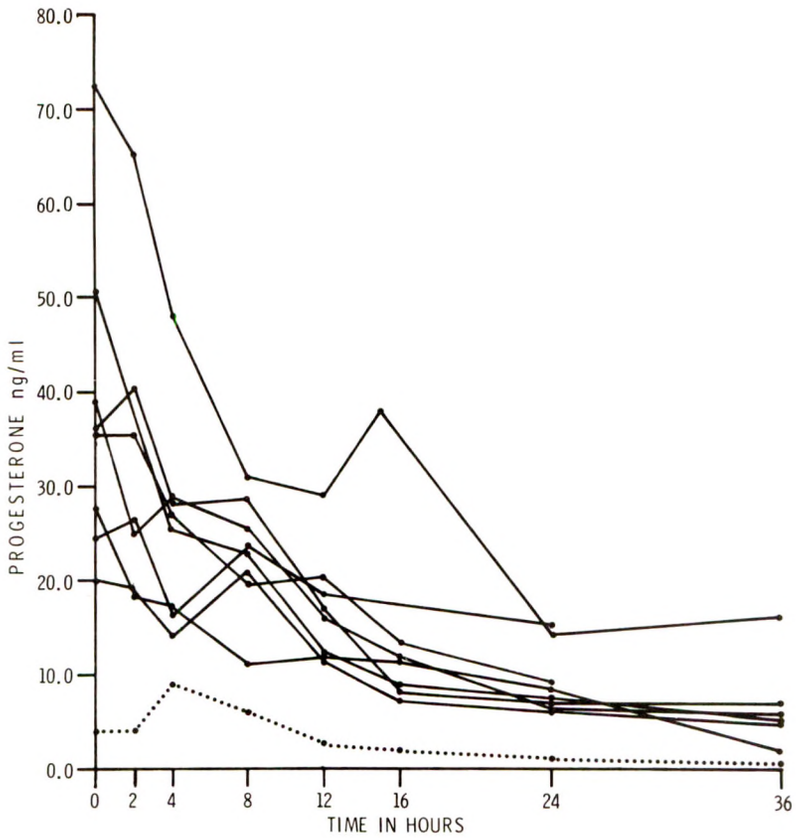


Fig. 1. The effect of serial intramuscular injections of 15-ME-$PGF_{2\alpha}$ for first-trimester abortion on the plasma progesterone levels in 9 patients. All patients were 5 to 6 weeks from the last menstrual period with positive pregnancy tests; however, the broken line represents a single patient presumed not to be pregnant at the time of 15-ME-$PGF_{2\alpha}$ administration. The first intramuscular injection of 15-ME-$PGF_{2\alpha}$ was administered at 0 hours.

A study was designed to examine the abortifacient effectiveness of serial intramuscular injections of 15(S)-15-methyl-$PGF_{2\alpha}$ (15-ME-$PGF_{2\alpha}$) in early gestations, 5 to 6 weeks after the last menstrual period. The effect of 15-ME-$PGF_{2\alpha}$ on plasma levels of progesterone, estradiol, human placental lactogen, and the β-subunit of human chorionic gonadotropin (HCG) was investigated.

Material and methods

Ten healthy women, ranging in age from 18 to 38 years, 5 to 6 weeks after the last menstrual period and with a positive pregnancy test (hemagglutination-inhibition test) volunteered to be included in this study. The patients were admitted for elective injections of 15-ME-$PGF_{2\alpha}$. An initial dose of 100 μg of 15-ME-$PGF_{2\alpha}$* was administered, followed in 60 minutes by 250 μg and 2 hours later by 250 μg. A dose of 250 μg of 15-ME-$PGF_{2\alpha}$ was then administered intramuscularly every 2 hours until the time of abortion or after 24 hours of therapy.

In order to minimize gastro-intestinal side effects, all patients were premedicated and continued on antiemetic and antidiarrheal agents throughout the abortion period as previously described.[3]

Blood samples were taken for determination of levels of plasma progesterone, estradiol, human placental lactogen, and the β-subunit of HCG prior to administration of 15-ME-$PGF_{2\alpha}$ and at 2, 4, 8, 12, 16 and 36 hours after the initiation of the procedure in 9 of the 10 patients. The patients were examined at regular intervals to determine the initiation of vaginal bleeding and to detect any signs of the products of conception in the vagina. Vital signs including heart rate, temperature, and respirations, were carefully monitored throughout the procedure. Twenty-two to 24 hours after the initial intramuscular injection of 15-ME-$PGF_{2\alpha}$ the patient underwent a careful internal examination followed by a suction aspiration with a 6 mm. Karman cannula. This procedure was performed to determine if the products of conception had been

*The 15-ME-$PGF_{2\alpha}$ was supplied by Dr. Edward Southern of the Upjohn Co.

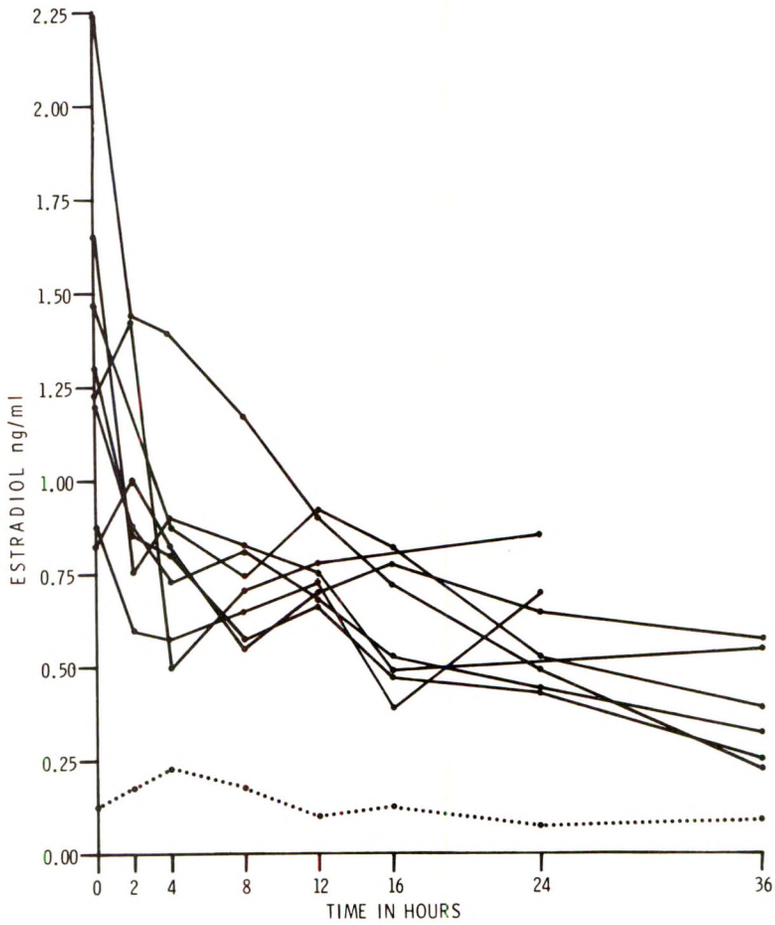


Fig. 2. The effect of serial intramuscular injections of 15-ME-PGF$_{2\alpha}$ for first-trimester abortion on plasma estradiol levels in 9 patients. All patients were 5 to 6 weeks from the last menstrual period with positive pregnancy tests; however, the broken line represents a single patient presumed not to be pregnant at the time of 15-ME-PGF$_{2\alpha}$ administration. The first intramuscular injection of 15-ME-PGF$_{2\alpha}$ was administered at 0 hours.

completely expelled. The tissue expelled through the vagina during prostaglandin administration was, when possible, collected and sent for pathologic analysis. The patients were seen for follow-up examination 7 to 14 days following the procedure.

Results

Eight of the 10 patients in this study with a history of amenorrhea of 5 to 6 weeks aborted completely with serial intramuscular injections of 15-ME-PGF$_{2\alpha}$. One patient started vaginal bleeding within 4 hours of the initial injection of 15-ME-PGF$_{2\alpha}$ but had aborted only incompletely by 24 hours when a suction completion could be performed without the need for anesthesia. One patient, 5 weeks after the last menstrual period and a positive hemagglutination-inhibition test on admission, responded to the prostaglandin administration with vaginal bleeding at 5 hours which continued for approximately 15 hours. A small amount of tissue was collected prior to surgical evacuation, but unfortunately this specimen was lost. On subsequent evacuation, no tissue was obtained; therefore, pathologic analysis was impossible. The patient's low hormone values of progesterone, estradiol, and HCG led us to believe that this patient was not pregnant at the time of the procedue. In the 8 successfully aborted patients, vaginal bleeding started from 1 to 8 hours after the initial intramuscular injection of 15-ME-PGF$_{2\alpha}$. The exact time of complete abortion cannot be calculated since there was a gradual and continuous passage of tissue during the period from the start of vaginal bleeding until the time of suction exploration of the uterine cavity, 22 to 24 hours after the start of prostaglandin administration.

When suction exploration was subseqeuently performed, the cervix was found to be soft and dilated, easily admitting a 6 mm. Karman cannula without the need for further dilatation and no tissue was obtained

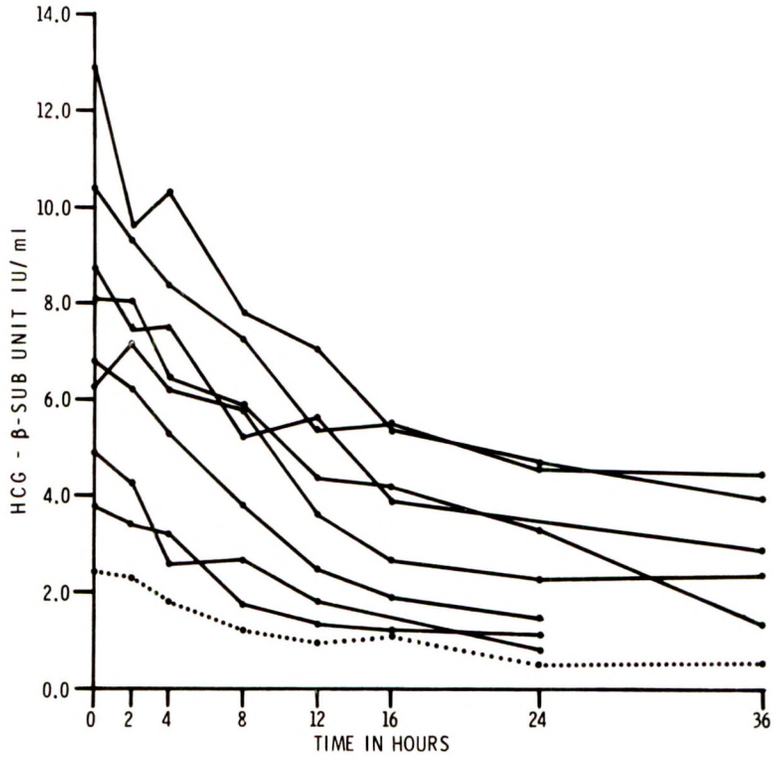


Fig. 3. The effect of serial intramuscular injections of 15-ME-$PGF_{2\alpha}$ for first-trimester abortion on the plasma levels of the β-subunit of HCG in 9 patients. All patients were 5 to 6 weeks from the last menstrual period with positive pregnancy tests; however, the broken line represents a single patient presumed not to be pregnant at the time of 15-ME-$PGF_{2\alpha}$ administration. The first intramuscular injection of 15-ME-$PGF_{2\alpha}$ was administered at 0 hours.

during the suction exploration in 9 patients. In the single incomplete abortion, the cervix was dilated but the products of conception had not been completely expelled.

Hormone levels are shown in Figs. 1 to 3.

Progesterone. Plasma progesterone levels dropped during the administration of 15-ME-$PGF_{2\alpha}$ (Fig. 1). There was no significant change at 2 hours after the initial prostaglandin injection, but there was a statistically significant difference of the mean value at 4 hours from the control period ($p < 0.01$). This drop in progesterone levels corresponds to the initiation of vaginal bleeding in the majority of patients. In the 8 pregnant patients studied, progesterone levels had dropped to 50 per cent of the control value at 8 hours after the initiation of therapy, 29 per cent at 24 hours, and 16 per cent at 36 hours. In the single nonpregnant amenorrheic patient, there was an initial rise in progesterone levels from the control value of 3.60 ng. per milliliter to a peak of 8.80 ng. per milliliter at 4 hours. In this patient, the progesterone then dropped over the treatment period to a level of 0.30 ng. per milliliter at 36 hours. In the patient with the incomplete abortion, the drop in plasma progesterone was compatible with the changes observed in the successfully aborted patients.

Estradiol. Estradiol levels also dropped during administration of 15-ME-$PGF_{2\alpha}$, and similarly to progesterone there was a statistically significant change from the control levels at 4 hours after the first intramuscular 15-ME-$PGF_{2\alpha}$ injection ($p < 0.01$) (Fig. 2). Estradiol levels dropped to 50 per cent of the control value at 16 hours and to 31 per cent at 36 hours. There was somewhat more fluctuation in individual levels of estradiol over the abortion period as compared to the steady fall observed in progesterone levels. In the single nonpregnant patient, estradiol levels rose initially and dropped minimally during prostaglandin administration.

***β*-subunit of human chorionic gonadotropin.** The level of the β-subunit of HCG dropped gradually and consistently in all patients during administration of 15-ME-$PGF_{2\alpha}$ (Fig. 3). Again, there was a statistically significant change from the control value at 4 hours ($p < 0.01$). Levels of the β-subunit of HCG were 50 per cent of the control value after 12 hours of administration of 15-ME-$PGF_{2\alpha}$ and 35 per cent at 24 hours and remained at 32 per cent at 36 hours. The

levels of HCG also dropped in the nonpregnant patient during prostaglandin administration.

Human placental lactogen. There was no consistent effect of administration of 15-ME-$PGF_{2\alpha}$ on levels of human placental lactogen, but the initial control levels of this hormone were very low.

Eight of the 10 patients in this series experienced gastrointestinal side effects during administration of 15-ME-$PGF_{2\alpha}$. These side effects were tolerable, and drug administration could be continued in all patients.

Comment

Abortion was successfully induced in 8 of 9 pregnant patients 5 to 6 weeks after the last menstrual period with serial intramuscular injections of 15-ME-$PGF_{2\alpha}$.

In the 9 pregnant patients, there was a fairly consistent drop in progesterone levels from those of the control period, which was significant at 4 hours after the first injection of 15-ME-$PGF_{2\alpha}$. However, uterine activity began within 15 to 30 minutes after administration of 15-ME-$PGF_{2\alpha}$ before any significant drop in progesterone was observed. It is interesting to note that in the pregnant patients vaginal bleeding began at about 4 hours in the majority of patients, at the same time that a significant drop in progesterone levels was observed.

Csapo and colleagues[4] reported on induction of abortion by luteectomy in a series of patients at less than 7 weeks' gestation. Seven of 11 patients were aborted successfully by this technique, and in these patients there was a drop in progesterone from a mean of 24.6 ng. per milliliter before luteectomy to 17.0 ± 3.3 ng. per milliliter at 24 hours after luteectomy. Okamura and co-workers[5] found no significant effect after $PGF_{2\alpha}$ infusion in 3 nonpregnant women when they examined the corpus luteum histochemically or electron microscopically. However, these authors found that administration of $PGF_{2\alpha}$ to a single pregnant patient was accompanied by some indication of luteolysis, suggested by the ultrastructure of the corpus luteum.

In our study there was also a significant drop in estradiol levels at 4 hours of prostaglandin administration. This drop in both progesterone and estradiol indicates that 15-ME-$PGF_{2\alpha}$ has a luteolytic effect since these two hormones are almost entirely produced in the corpus luteum in this early stage of gestation. However, it is not certain if the luteolysis was the precipitating factor in the mechanism of abortion. The high, unphysiologic doses of 15-ME-$PGF_{2\alpha}$ initiated strong uterine activity very rapidly, and the persistent uterine activity may have played the major role in abortion of these patients In the study of Csapo and colleagues,[4] abortion did not occur for several days after the luteectomy in early pregnancies, while in our series abortion was complete in 8 of 9 patients within 24 hours. The drop in the β-subunit of HCG in this study is indicative of placental damage related to prostaglandin administration, but the exact time of this effect cannot be ascertained since the hormone is excreted very slowly.

In all patients in this study, administration of 15-ME-$PGF_{2\alpha}$ was accompanied by a gradual softening and dilatation of the cervical os which permitted surgical exploration of the uterus without the need for mechanical dilatation. Administration of intramuscular 15-ME-$PGF_{2\alpha}$ may have some clinical usefulness in cases where a gradual softening and dilatation of the cervix is desired before suction evacuation.

This is in agreement with the findings of Dingfelder and associates,[6] who found that $PGF_{2\alpha}$ vaginal suppositories reduced cervical resistance when placed intravaginally 3 hours prior to suction abortion.

We wish to thank the professional staff of the abortion unit, The New York Hospital, for their valuable assistance. We also wish to thank Dr. Joseph T. Sabota, Clinical Research Laboratories, The Upjohn Co., for the hormone studies, and Glenn Gutknecht and Louis Piccoli, The Upjohn Co., for their support of this project.

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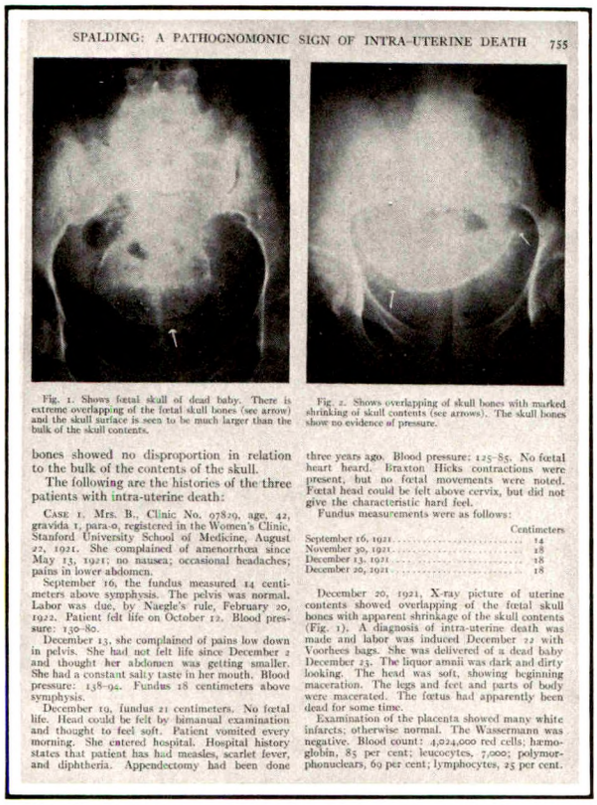
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Classic pages in
OBSTETRICS AND GYNECOLOGY
Lawrence D. Longo, M.D.

A pathognomonic sign of intra-uterine death

ALFRED BAKER SPALDING

Surgery, Gynecology and Obstetrics, vol. 34, pp. 754-757, 1922



SPALDING: A PATHOGNOMONIC SIGN OF INTRA-UTERINE DEATH 755

Fig. 1. Shows fœtal skull of dead baby. There is extreme overlapping of the fœtal skull bones (see arrow) and the skull surface is seen to be much larger than the bulk of the skull contents.

Fig. 2. Shows overlapping of skull bones with marked shrinking of skull contents (see arrows). The skull bones show no evidence of pressure.

bones showed no disproportion in relation to the bulk of the contents of the skull.

The following are the histories of the three patients with intra-uterine death:

CASE 1. Mrs. B., Clinic No. 07829, age, 42, gravida 1, para-0, registered in the Women's Clinic, Stanford University School of Medicine, August 22, 1921. She complained of amenorrhœa since May 13, 1921; no nausea; occasional headaches; pains in lower abdomen.

September 16, the fundus measured 14 centimeters above symphysis. The pelvis was normal. Labor was due, by Naegle's rule, February 20, 1922. Patient felt life on October 12. Blood pressure: 130-80.

December 13, she complained of pains low down in pelvis. She had not felt life since December 2 and thought her abdomen was getting smaller. She had a constant salty taste in her mouth. Blood pressure: 138-94. Fundus 18 centimeters above symphysis.

December 19, fundus 21 centimeters. No fœtal life. Head could be felt by bimanual examination and thought to feel soft. Patient vomited every morning. She entered hospital. Hospital history states that patient has had measles, scarlet fever, and diphtheria. Appendectomy had been done three years ago. Blood pressure: 125-85. No fœtal heart heard. Braxton Hicks contractions were present, but no fœtal movements were noted. Fœtal head could be felt above cervix, but did not give the characteristic hard feel.

Fundus measurements were as follows:

	Centimeters
September 16, 1921	14
November 30, 1921	18
December 13, 1921	18
December 20, 1921	18

December 20, 1921, X-ray picture of uterine contents showed overlapping of the fœtal skull bones with apparent shrinkage of the skull contents (Fig. 1). A diagnosis of intra-uterine death was made and labor was induced December 22 with Voorhees bags. She was delivered of a dead baby December 23. The liquor amnii was dark and dirty looking. The head was soft, showing beginning maceration. The legs and feet and parts of body were macerated. The fœtus had apparently been dead for some time.

Examination of the placenta showed many white infarcts; otherwise normal. The Wassermann was negative. Blood count: 4,024,000 red cells; hæmoglobin, 85 per cent; leucocytes, 7,000; polymorphonuclears, 69 per cent; lymphocytes, 25 per cent.

The roentgenographic evidence of fetal death, overlapping of skull sutures in the absence of labor, was described almost simultaneously by two gynecologists working independently. Spalding, of Palo Alto, California, wrote, "It seems that very shortly after intra-uterine death, the brain tissue shrinks, which produces a typical overlapping of the foetal skull bones. This . . . gives a picture quite different from the overlapping produced by molding. With the x-ray, the decreased size of the foetal head from post-mortem shrinking can be determined because the cranial bones remain nearly the same size and shape. As the head shrinks, the bones overlap at times to an astonishing distance. The radius of curvature of the shrunken head becomes obviously smaller than that of the unchanged cranial bones." He presented three case reports of fetal death. In the succeeding issue of the same journal (Surg. Gynecol. Obstet. 35: 67, 1922), in a paper on roentgenography in obstetrics, D. A. Horner of Chicago briefly described the same sign. He noted, ". . . during the past year in three cases where foetal death was suspected, x-ray revealed overriding of the skull bones with asymmetry of the head. This to me indicated foetal death and the subsequent clinical course bore out my belief." The sign may be seen as early as a week following death.

COMMUNICATIONS IN BRIEF

This section is suitable for reporting cases, results of therapeutic trials, and descriptions of new procedures or instruments. Reports should be limited to seven hundred words and two references. Use of an illustration or table requires a proportionate reduction in total words.

Amelanotic melanoma metastatic to the placenta

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ALTHOUGH maternal cancer may occur as frequently as one in 1,000 pregnancies, metastasis to the placenta and/or fetus is exceptionally rare. Of the 28 reported cases of maternal cancer metastasizing to the products of conception, 13 have been malignant melanomas.[1, 2] Of these 13, six have been reported to involve only the placenta, five involved the fetus, and two involved the fetus and placenta. The rarity of this occurrence, despite the relative frequency of the concurrence of cancer and pregnancy, has given rise to suggestions in the literature that more attention must be paid to the biologic significance of cancer in pregnancy and that all cases of metastatic disease to the products of conception warrant reporting in the literature.[2]

It is the purpose of this paper to present the ninth documented case of malignant melanoma metastatic to the placenta and the first in which such a tumor has been amelanotic. Additionally, this report stresses appropriate methods of study of such cases.

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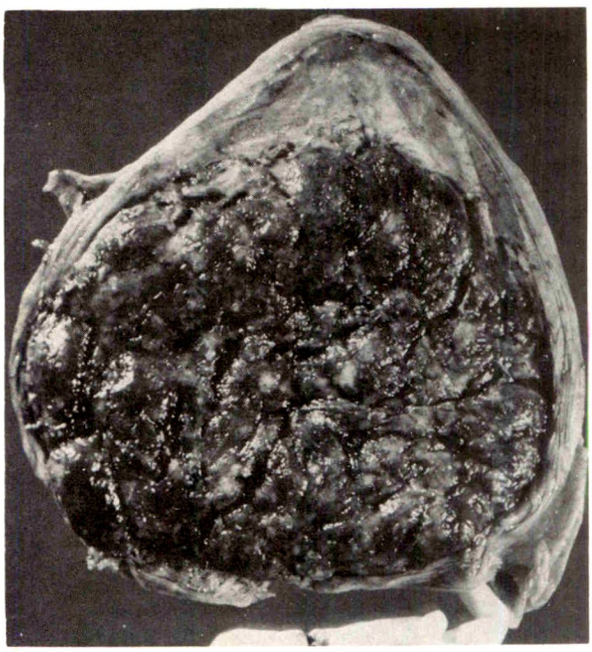


Fig. 1. Maternal surface of the placenta. The white nodules are metastases of amelanotic melanoma, but could easily be mistaken for placental infarcts.

A 43-year-old woman, gravida 5, para 1, abortion 3 at 30 weeks' gestation, had had a malignant melanoma widely excised from her midback 4 years previously. The patient was otherwise entirely healthy and did well until 18 weeks' gestation, when a radical right axillary dissection was performed for recurrent tumor. The patient decided to continue the pregnancy.

Over the succeeding 12 weeks, she developed rapidly increasing dyspnea, cough, and right chest pain, requiring narcotics for relief. Chest x-rays showed diffuse pulmonary and pleural densities, occupying almost the entire right hemithorax, and mediastinal widening.

The patient was admitted to the hospital for early delivery, since maternal survival until term was considered unlikely and institution of antitumor chemotherapy was considered mandatory. At 31 weeks' gestation, cesarean section with bilateral partial salpingectomy was performed. Amniotic fluid was colorless and no tumor nodules were present in the liver or on any peritoneal surfaces. Postoperatively, the patient became ambulatory with only mild dyspnea, but severe right chest pain persisted. She was discharged on the eighth postpartum day. Despite the institution of antitumor chemotherapy, the patient's condition deteriorated rapidly and she died on the fifty-fourth postpartum day, in what would have been the thirty-eighth week of gestation. There was clinical evidence of widespread metastatic disease, but permission for autopsy could not be obtained.

The 1,180 gram female infant had no clinical evidence of metastatic melanoma. From 3 hours of life, she developed respiratory distress. Despite mechanical respiratory assistance and vigorous supportive therapy, the infant died on the second day of life. At autopsy, there was no gross or microscopic evidence of melanoma. Death was judged to be related to persistent fetal circulation, hyaline membrane disease, and pulmonary and subarachanoid hemorrhage.

The cord and 3 chorioamniotic membranes were entirely normal. As shown in Fig. 1, the maternal surface of the placenta revealed multiple firm white nodules which were approximately 0.5 cm. in diameter and grossly resembled infarcts seen in term placentas. Histologically, they were located midway between the decidua and chorionic plate and consisted of septa, villi, and insinuating groups of cells forming a syncytia, sometimes adjacent to recently necrotic placental tissue and fibrin deposits. Microscopic invasion of villi by tumor was identified. Cells interpreted as tumor cells superficially resembled cytotrophoblasts, but were approximately twice as large. The nuclei were pleomorphic and had prominent nucleoli. A few tripolar mitoses were found. The cytoplasm rarely contained definite melanin granules. Histochemical staining for the dopa reaction, for melanin precursor identification, was positive in the cells which corresponded to those cells described above as tumor cells. Tissue culture of cells from the nodules was attempted, but no dopa-positive cells were present after two transfers.

Metastatic disease to the products of conception has been reviewed as recently as 1972 in the German[1] and in 1970 in the English language literature[2] and this will not be repeated in depth here. In brief, melanomas account for approximately one half of all reported cancers metastatic to the products of conception, whereas they make up only 8 per cent of malignancies occurring in conjunction with pregnancy. Typically, these melanomas have developed prior to or during early pregnancy, have metastasized widely, and eventually produced death, as in this case.

Various methods of investigation of these cases have been suggested, including, for example, cytologic examination of maternal and umbilical cord blood[2] and definition of immunologic factors; however, the method of prime importance remains the careful examination of the placenta. The gross appearance of the placenta may be entirely normal in the presence of metastatic disease. In this case, the placental nodules could easily have been mistaken for infarcts, were it not remembered that the source was a 31 week, rather than term, pregnancy. Histologic examination clarified the nature of the nodules.

Melanoma has not been reported to have metastasized to the fetus without placental involvement. Invasion of the villi has been evaluated in seven cases, including this one. No invasion was present in two cases and both infants survived. Of five infants whose placental villi were invaded, two died with metastases, two died without metastases, and one survived. Thus, determination of the presence or absence of invasion of the placental villi appears to be of importance, since the results may have prognostic significance for the infant.

The fact that the melanoma cells metastatic to the placenta in this case were amelanotic presented a special diagnostic problem. Tissue culture of tumor cells was attempted, but was not successful; however, histochemical staining of microscopic sections for the dopa reaction allowed the tumor cells to be differentiated from cytotrophoblasts and to be identified definitely as melanoma cells.

This case suggests that when pregnancy and maternal malignancy coexist, appropriate investigations should include careful gross and microscopic examination of the placenta with particular attention to the presence or absence of invasion of the villi and may include the use of special techniques, such as histochemical staining and tissue culture.

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Self-limited acute defibrination in pregnancy: Case report

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DEFIBRINATION during pregnancy is commonly associated with amniotic fluid embolism, abruptio

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Table I. Hematologic findings

	*Time from delivery**				
	−6 hr.	*−3 hr.*	*0*	*+6 hr.*	*+18 hr.*
Hematocrit *(%)*	35	35	29	23	28
Platelets	Decreased	58,000	70,000	102,000	112,000
Fibrinogen (mg./100 ml.)	Nondetectable	0.04	0.06	0.13	0.3
Fibrinogen titer	—	1:2 no lysis	1:8	—	1:64
Fibrin split products (μg/ml)	—	640-1,280	—	—	20-40

*Transfused 2 units of packed cells and 2 units of fresh frozen plasma at +12 hours.

placentae, and retention of a dead fetus. Sepsis, profuse hemorrhage, or transfusion of mismatched blood may also initiate the process. We present here a patient who developed acute onset of self-limited defibrination in late pregnancy not clearly associated with any of these disorders.

Y. E., a 27-year-old woman, gravida 3, para 3, presented at the LAC/USC Medical Center at 37 weeks' gestation. Her prenatal course had been unremarkable until the evening of admission, when she was awakened with an acute episode of sharp midabdominal pain which lasted for about 1 minute and was replaced by a cramping abdominal pain which lasted for perhaps half an hour and which the patient thought represented contractions. Coincident with the lower abdominal pain the patient experienced acute onset of shortness of breath, substernal discomfort, and transient cyanosis as observed by her husband. All symptoms subsided within 1 hour. At no time did she notice vaginal bleeding or escape of fluid from the vagina or have other complaints.

Physical examination, 1 hour later, revealed a well-nourished, well-developed woman in no acute distress. The blood pressure was 120/70 mm. Hg, the pulse was 78 and regular, and the temperature was 98.8° F. The chest was clear to percussion and auscultation and the cardiac size was normal. The abdomen was nontender; the uterine fundus measured 40 cm. and was soft and nonirritable. The fetus was easily palpable and estimated to weigh 7½ pounds. Pelvic examination revealed the cervix to be 50 per cent effaced, 2 cm. dilated, a normal vertex at −1 station. The membranes were intact. The fetal heart rate, as determined by auscultation, was 132 beats per minute and regular.

Routine venipuncture revealed nonclotting blood both in the test tube and at the venipuncture site. The hematocrit was 32 per cent; examination of the peripheral smear revealed no platelets. Results of hematologic examination of blood specimens taken at admission and subsequently are presented in Table I. No red blood cells were found in the urine and the chest x-ray was unremarkable.

The diagnosis of consumption coagulopathy with defibrination, presumably secondary to either abruptio placentae or amniotic fluid embolism, was made and the patient was taken to the labor suite. Preparations were made for possible cesarean section which included the availability of fresh frozen plasma, platelets, packed cells, and factor VIII–rich cryoprecipitate. External monitoring at this time revealed a stable fetal heart rate about 140 b.p.m.; contractions were mild, occurring every 5 to 10 minutes. Amniotomy was performed which revealed blood-tinged (hematocrit, 6 per cent) but otherwise clear amniotic fluid. Internal fetal monitoring was begun. The fetal heart rate pattern revealed multiple premature supraventricular extrasystoles, but no other evidence of fetal distress. Over the next 2 hours, the patient failed to progress in labor and oxytocin was begun.

This was followed by rapid progress in cervical dilatation; 4½ hours later there was uneventful, spontaneous vaginal delivery of a 3,400 gram male infant who scored Apgar 8 and 9 at 1 and 5 minutes, respectively. The placenta was delivered 5 minutes after delivery, weighed 450 grams, and showed no evidence of retroplacental clots. There were no pelvic or perineal lacerations. The patient had a transient episode of uterine atony which responded well to oxytocin and fundal massage. The total estimated blood loss was 800 ml.

The infant's neonatal course was unremarkable. The arrhythmia disappeared shortly after birth. The mother's postpartum course was uneventful. Urine output was consistently good and there was no evidence of further bleeding or hemolysis. The hematocrit equilibrated to 24 per cent by the day after delivery, for which the patient received 2 units of packed cells and 1 unit of fresh frozen plasma. Lung scan, obtained on the first postpartum day, revealed a filling defect in the right base consistent with pulmonary embolism. Chest x-ray and blood gases were within normal limits. In the absence of any clinical findings of abruptio placentae, a diagnosis of defibrination secondary to *forme fruste* amniotic fluid embolus was made.

Defibrination in obstetric patients has been found in association with abruptio placentae, amniotic fluid embolism, sepsis, retained dead fetus, profound hemorrhage, and shock.[1] This patient presented with little to suggest any of these diagnoses. Nor was her pregnancy complicated by various factors which have been considered to predispose to amniotic fluid embolism, namely multiparity, tumultuous labor, meconium, ruptured membranes, abruptio placentae, marginal previa, and oxytocin. In most series, however, approximately 10 per cent of patients have presented prior to onset of labor. The short-lived symptoms suggest to us that a transient, self-limited episode of amniotic fluid embolism is the most likely diagnosis.

The severe episode of abdominal pain which preceded the pulmonary symptoms is consistent with the diagnosis of spontaneous uterine hyperactivity or tetany.

Amniotic fluid embolus was first described in patients developing cyanosis, dyspnea, sudden shock, and death. Later reports included patients who survived the initial insult only to die in or after labor with severe bleeding diathesis due to low concentration of circulating fibrinogen.

Gregory and Clayton[1] described filling defects in lung scans in two patients found to have amniotic fluid embolism, one of whom survived. Positive lung scans in patients with consumption coagulapathy might also result from primary clotting in the lung rather than embolization, as shown by studies of the Birmingham Eclampsia Study Group.[2]

We believe the present case represents a *forme fruste* case of amniotic fluid embolism arising in a multigravid woman before the onset of labor. It is tempting to speculate that the severe abdominal pain which awakened the patient was, in fact, a spontaneous tetanic contraction which caused an extrusion of amniotic fluid or trophoblastic tissue first into the uterine sinuses, then the central circulation, and ultimately transported to the lungs. The patient demonstrated marked fibrinogen depletion which was self-limited and, in fact, recovering during the induction of labor. We hope this report will stimulate the documentation of similar cases.

Special thanks are due to Dr. Ezra Davidson for his comments on the paper.

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Two new laparoscopic instruments: Bipolar sterilizing forceps and uterine manipulator

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THE ELECTROSURGICAL hazards associated with laparoscopic surgical procedures are both theoretical and real. The use of unipolar instruments in which the bulk of the patient is included within the circuit introduces spread of high-frequency radio waves into tissues beyond those in the immediate operating field. That this theoretical risk is in reality a practical concern is evidenced by the finding in a recent survey of an incidence of reported accidental bowel burns of approximately one per thousand laparoscopic sterilizations.[1] Not included in this figure were burns to other sites as well as injury to the operator. The exact mechanism of this type of injury has not been elucidated. Experienced surgeons have reported cases of burns in which all instruments were insulated, and isolated (to ground) solid-state generators were used.

Our interest in this area prompted development of a bipolar instrument for tubal coagulation and severance under laparoscopic guidance.[2] In a bipolar circuit current passes through only the tissue in proximity to the two sides of the instrument, i.e. between the jaws.

Prerequisites were as follows: (1) the instrument must be sturdy; (2) it must be capable of mechanically cutting, if desired, but must not be so sharp as to cause bleeding by traction alone; (3) it must be used with a low-output generator.

The instrument which evolved after various prototypes had been employed is shown in Fig. 1. It was developed in cooperation with Mr. John Martin of the Cameron Miller Instrument Company, (Chicago, Illinois), and was designed around the Model 7910 generator manufactured by this company, although it can be used with other similar solid state generators.

It has the following salient features: (1) Cutting is accomplished mechanically with guillotine-like jaws. (2) The handle is adjustable to allow the jaws to rotate around the long axis for operator convenience. (3) The shaft is insulated internally to avoid external insulation wear and possible deposition of foreign material within the abdomen. (4) The key feature is the development of suitably applied insulation between the jaws of the instrument. Because of close electrical and mechanical tolerances, this instrument has a minimum diameter of 5 mm., permitting use through a second-incision technique. It will not fit through an operating scope channel. Work is currently under way to provide a 4.5 mm. longer instrument for a one-puncture approach. (5) Paradoxically, grasping more tissue and holding it tighter produces a reduction in tissue electrosurgical effect. Proper technique calls for a very light touch. Because of the discrete lesion produced, it is necessary to "march" across the Fallopian tube in two or three applications to adjacent points. With a generator setting designed to deliver approximately 35 watts, damage to the collateral circulation within the broad ligament is eliminated. The "starburst" lesion within the mesosalpinx commonly seen with unipolar instruments has not been encountered.

The author has no financial interest in the sale of instruments described herein.

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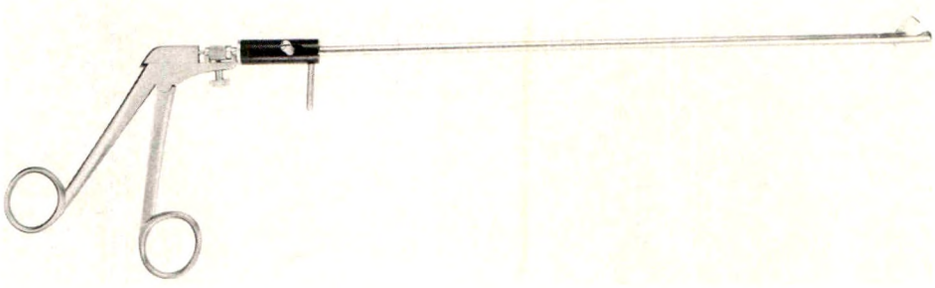


Fig. 1. Bipolar instrument for sterilization.

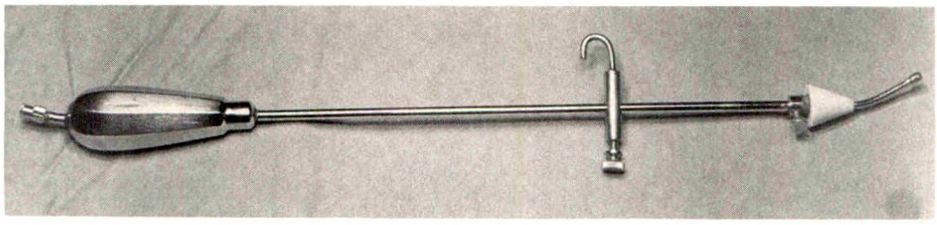


Fig. 2. Weighted intrauterine manipulator and cannula.

Prior to release for commercial use, instruments were circulated to a number of independent laparoscopists, who reported favorable results. The present model has been employed in daily use at the Pennsylvania Hospital for a period of 13 months. During this interval, approximately 600 sterilizations were performed by numerous operators with no untoward electrosurgical effect noted. Two mechanical faults resulting in instrument repair were recognized and were eliminated by changes in design and fabrication.

Bipolar laparoscopic instruments offer advantages over unipolar equipment. First, the need for a ground or return electrode is eliminated. Thus, a grounding plate as well as the functional integrity of that part of the electrical connection is eliminated. Further, the risk of accidental burns at distant sites is avoided. The discrete nature of the lesion may cause less havoc within the vascular arcade of the broad ligament. This advantage may be of particular importance since interference with this circulation may cause ovarian dysfunction. There has been nagging doubt for many years, (never proved) that some women do indeed have menstrual aberrations following sterilization procedures which interfere with the ovarian-uterine collaterals. We are about to undertake a study of this problem and hope to generate significant data.

Laparoscopic visualization of a uterine sound as it is pushed through the fundus by a well-meaning assistant is disconcerting for the surgeon and potentially damaging to the patient. Actually, correct manipulation calls not for a vector force upward but rather a downward pull on the uterus with anterior rotation about the cardinal ligaments. This causes the uterus to roll forward toward the anterior abdominal wall with better exposure of the adnexal and cul-de-sac structures.

The device shown here has simplicity of design and the absence of springs, pumps, and the like (Fig. 2).

The hollow handle is ballasted with 10 oz. of weight in order to produce the desired pull and rotation. A standard female fitting allows syringe attachment to execute tubal lavage.

The distal end of the instrument features a malleable tip for uterine insertion. The tip is only slightly flared to eliminate the need for preliminary cervical dilatation except in the presence of cervical stenosis, yet lessen the chance of uterine perforation during insertion. An adjustable Teflon collar provides a cervical stop and seal and is not damaged by autoclaving or immersion in various soaking solutions. An adjustable hook permits the manipulator to be held to the cervix by locking it to the handle of any standard uterine tenaculum.

The only maintenance necessary is flushing the device with water after each case to remove pieces of endometrium or clots which may have accumulated at the tip. The patient must be positioned in a dorsal lithotomy position with the buttocks at the end of the table so that the instrument can hang freely, usually at a 60 degree angle to the horizontal. If a posterior vaginal speculum is used to visualize the cervix, it should be removed after insertion of the manipulator. The operator may use one hand passed over the pubis to move the manipulator himself or may have an

assistant move it from side to side. Motion in a cephalad direction is unnecessary unless the uterus is being used as a piston to remove small bowel from the pelvis.

The instrument is helpful for adnexal visualization during sterilization procedures or for diagnostic cases, especially those employing tubal patency tests. It can also be used to advantage for manipulation of the uterus and adnexa for laparotomy via a "mini lap" incision.

This piece of equipment can be made easily by those handy with a spot welder or purchased from John Marco and Sons (Oakhurst, New Jersey).

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A new self-retaining vaginal retractor

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GOOD EXPOSURE is essential for ease and precision in surgical operations. While the problem of exposure has been satisfactorily solved in most operations, there has been little progress in the field of vaginal surgery. Customarily two or three hands are tied up with retraction so that, even with two assistants, the gynecologic surgeon is left with inadequate first assistance. Generally the Auvard weight speculum is used for the posterior wall and perineum and two lateral retraction blades are hand held.

In 1933 White[1] devised a self-retaining vaginal retractor with side blades attached to the wings of the Auvard speculum. The mechanical means of spreading the blades was limited and the instrument was not generally adapted. In 1935 Picto,[2] with the Auvard speculum as a base, used wing nuts and bolts to hold and fix the lateral blades. The difficulty with this retractor was the rigidity of the retracting blades on the base, so that the edges of the blades would not adjust to various pubic angles. In addition, the use of nuts as a means of holding retractors tended to allow slippage.

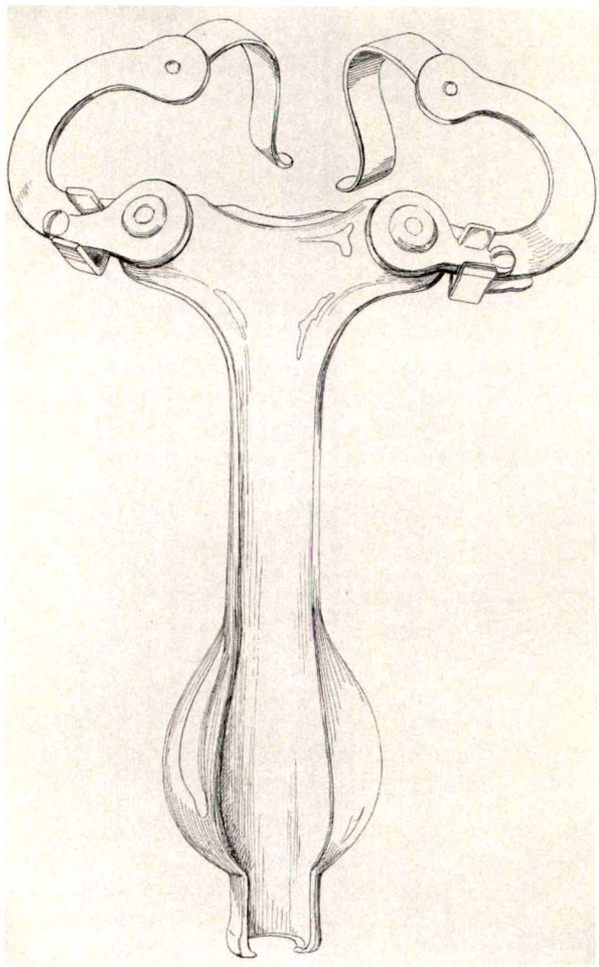


Fig. 1. New self-retaining vaginal retractor.

The O'Sullivan-O'Connor self-retaining vaginal retractor allowed the lateral retractor blades to swivel, which was an improvement, but, by not using a weighted speculum as a base, the retractor was unstable, since it depended on four-sided pressure of its blades on the vaginal walls to stay in place. Anterior colporrhaphy was prevented by the anterior blade.

Essentially there has been no progress in development of a suitable self-retaining vaginal retractor until about 9 years ago when the Wexler retractor,* here presented, was devised (Fig. 1). The Auvard weighted speculum was used as a base because of its stability and familiarity to all gynecologists. To this, two lateral retractors were made easily attachable to the wings by

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*Manufactured by Edward Weck & Company, Inc., 49-33 31st Place, Long Island City, New York 11101.

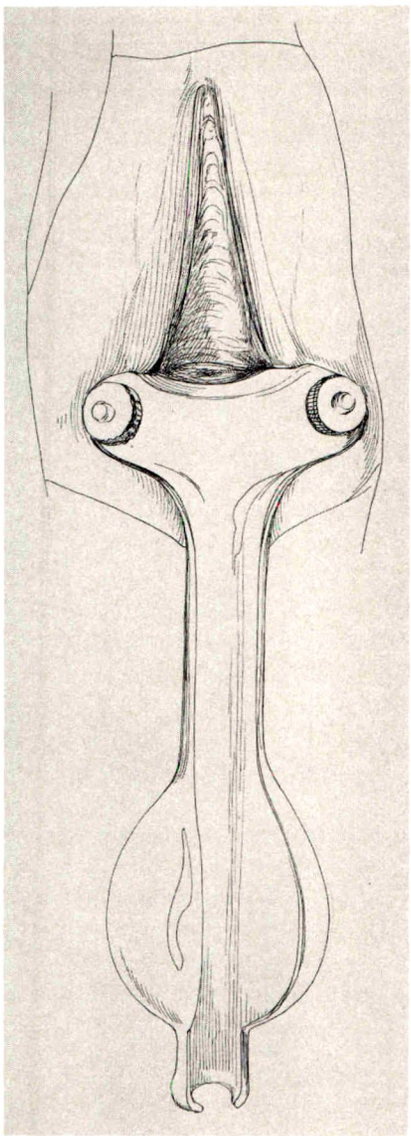


Fig. 2. Weighted speculum in position. Notice the triangular visibility.

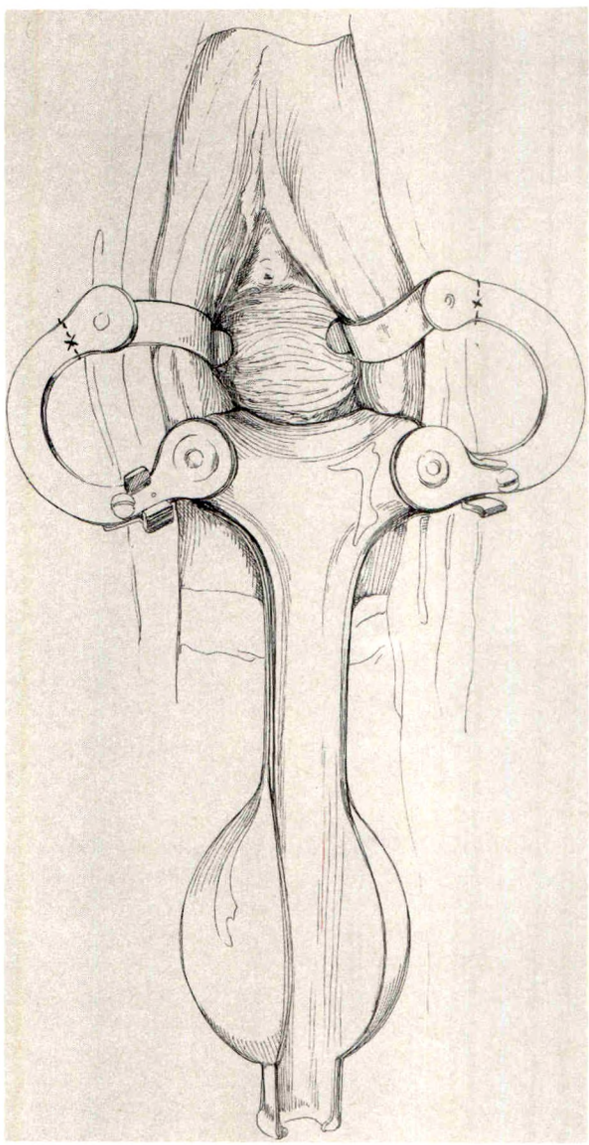


Fig. 3. New vaginal retractor with side blades inserted and retracted, creating a wide rectangular area of visibility.

means of a set of continuously adjustable ratchets. The blades were swiveled so they would adjust to the individual pubic arch. The entire assembly was made of stainless steel. In use the weighted speculum is inserted first, then the side blades are attached and retracted.

Fig. 2 shows the visibility when only the Auvard retractor is used. Note that the exposure is a triangle.

Fig. 3 shows the Wexler self-retaining vaginal retractor in place. The exposure is a large rectangle.

The advantages of this retractor are as follows. (1) Exposure is increased without hand-held retractor blades. (2) A secure and easily attached and adjustable ratchet for retraction of the side blades is provided. (3) The lateral blades swivel, to adapt easily to the pubic angle. (4) A stainless-steel Auvard weighted speculum provides a firm base. (5) Gynecologic surgeons using only the Auvard speculum customarily sew back the labia for increased exposure. This is unnecessary with the retractor described.

In patients with a shortened vagina due to a marked prolapse, all retractors tend to fall out when the cervix is pulled forward. When this happens, two sutures placed as indicated in Fig. 3 will hold the retractor in place. The retractor is removed for operations on the posterior wall and is replaced by a Guelpi retractor.

With the Wexler retractor only a single hand is occasionally needed for retraction of the anterior

vaginal wall or the open vaginal vault in a vaginal hysterectomy. The first assistant can generally have both hands free to assist the surgeon.

The Wexler retractor has been used at Southside Hospital for 9 years. All necessary modifications have been made. It is our opinion that when used for anterior colporrhaphy, cold cone of the cervix, or vaginal hysterectomy it will be a welcome addition to the armamentarium of the gynecologic surgeon.

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CORRESPONDENCE

Epidural analgesia

To the Editors:

In the paper "Epidural analgesia for obstetrics" (AM. J. OBSTET. GYNECOL. 120: 1055, 1974), McDonald and associates suggest practices which are not supported by the data presented. In particular, the recommendation to avoid regional analgesia in the presence of abnormal fetal heart rate parameters or antepartum evidence of placental insufficiency is not upheld by their evidence.

The authors criticize another recent study on epidural analgesia because ". . . no group treated with other analgesic methods was offered as a comparison." This criticism applies to their own work. Each of their patients acted as her own control, and we would speculate that any changes might be part of an ongoing process. Indeed, 32 of the 41 patients studied (Croups A and B) had late deceleration patterns during the 30 minute period prior to the onset of epidural anesthesia. It seems that these investigators have simply corroborated that fetuses with uteroplacental insufficiency show greater changes as labor progresses, regardless of the form of anesthesia used. In addition, even the increase in late decelerations found in Group A during epidural anesthesia may be the result of random changes, that is, no statistical significance can be attributed to this change, probably because the changes were small and variable and because a sizable fraction of the remaining abnormal population (Group B) showed no change or a decrease in late decelerations.

In their study, nine patients (Group C) had no abnormal patterns before administration of epidural anesthesia. None of these patients developed abnormal patterns during anesthesia, which is in agreement with other studies. Belfrage and colleagues[1] monitored the fetal heart rate (FHR) and fetal scalp acid-base status in 37 patients with uncomplicated labor who received epidural anesthesia. They found no abnormal FHR tracings or pH values during blockade. Similarly, Zador and Nilsson[2] found that only one of 22 patients who received epidural anesthesia and did not have hypotension developed late deceleration patterns, and these were sporadic and transitory.

The innocuousness of epidural analgesia is further suggested by the Apgar scores and acid-base statuses from the infants in the study of McDonald and associates. No significant differences were found among these values from the different groups. Furthermore, the average Apgar scores for all groups were within the normal (7-10) range and by 5 minutes were 8.5 or greater. Thus, the suggestion of McDonald and associates to ". . . be generous in restricting regional analgesia. . ." is unwarranted. In fact, the opposite recommendation could be made. Their evidence indicates that: (1) Normal patients given epidural anesthesia do not display fetal acidosis or late deceleration; (2) no neonatal depression, as evidenced by Apgar score or umbilical cord acid-base status, can be attributed to epidural anesthesia, even in the presence of maternal or fetal complications; (3) epidural anesthesia appears to benefit the infant of a toxemic mother, as evidenced by unusually good Apgar scores (8 and 9 at one and five minutes, respectively).

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Reply to Drs. Levinson and Shnider

To the Editors:

Drs. Levinson and Shnider stated that we suggested practices not supported by the data presented and that specifically the recommendation to avoid regional analgesia in the presence of abnormal fetal heart rate parameters or antepartum evidence of placental insufficiency is not upheld by our evidence. A careful examination of the next to the last paragraph should clarify this point. In that paragraph, we stated that this study revealed no serious intrauterine asphyxia after administration of a lumbar epidural analgesic agent in instances of *mild* late deceleration (all the late decelerations in our study happened to be of the defined *mild* variety). Nevertheless, we wish to point out that this should not be taken as a blanket statement and we would still suggest restriction of regional analgesic

agents when two conditions exist. Both of the conditions, as outlined in that paragraph, bespeak of what most obstetricians would consider an ominous threat to fetal reserve for labor. These conditions are: (1) a flat heart rate and moderate to severe late deceleration, and (2) antepartum evidence of reduced placental reserve as depicted by low estriol values and a positive oxytocin challenge test. No such patients as just outlined have been studied in any paper to date, to our knowledge. Furthermore, who would be so presumptuous as to want to administer regional analgesic agents in such a case when they know full well the threat of a reduced placental reserve might be life threatening to the fetus if the most common complication of regional analgesia—hypotension—should occur? Thus, a closer look at their accusation, that we suggested a practice not supported, reveals that what we were actually doing was placing the spectrum of fetal reserve into perspective so that readers would not think that obstetric regional analgesic agents are completely devoid of restrictions. We were not suggesting and did not promote its restriction in cases such as those studied where only mild late deceleration was evident.

A second point of disagreement is that we had no control group in our study. The intent of this study was to utilize lumbar epidural analgesia under the most optimal conditions and monitor and follow closely all patients in a truly specialized setting. We wanted to see if segmental lumbar epidural analgesia with minimal drug exposure and hypotension in the presence of mild late deceleration would be injurious to the fetus. This was because at the time this study was underway the accusation that lumbar epidural analgesia is not safe in such instances was clearly inferred by many obstetricians. We believe this objective was achieved satisfactorily. It was our intent to complete another separate study with an established control group. Shortly after the study was completed in 1973, we began, and have now completed, the control group study which we hope to have published soon.

A third point argued was that we simply corroborated that fetuses with uteroplacental insufficiency show greater changes as labor progresses, regardless of the form of anesthesia. The correct terminology should be "analgesia" since anesthesia is not used for relief of first-stage discomfort. This is definitely not correct since we did not study patients with multiple varieties of analgesia.

We appreciate that they formed the opinion lumbar epidural analgesia is relatively innocuous but, as stated earlier, to translate this to a sweeping statement, ". . . that restricting regional analgesia is unwarranted . . ." is hardly logical in lieu of the aforementioned defined circumstance which we reoutlined here for such a restriction.

Just so no one will accuse us of acquiescing with their three summary points, we would make some amendments: For Point No. 1, "if hypotension does not occur" should be appended at the end of the sentence. For Point No. 2, "as defined by mild late deceleration in labor" should be appended to the end of the sentence. The third statement is true, but a larger control study must be reported before any such assumption could be acceptable.

Since our study was published, two subsequent studies have similarly reported that segmental lumbar epidural analgesia does not have a deleterious effect upon the fetal heart rate and acid-base balance of the fetus.[1, 2] We hope that our original study, these two subsequent studies, this reply to the Letter to the Editors, and our control study, soon to be published, will all help to alleviate the apprehension that some obstetricians and anesthesiologists have in regard to lumbar epidural analgesia for labor and delivery. We believe this remains the optimum technique for labor analgesia and still affords the greatest flexibility and is the most effective method for maternal comfort during labor. Nevertheless, we must continue in our effort to study all aspects of any analgesic method to ascertain that fetal and neonatal jeopardy are minimized since that must, in the final analysis, be the governing restriction for any method.

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Obstetric anesthesia in New York City

To the Editors:

In April, 1974, Dr. Jean Pakter, Director of Maternal and Child Health of the City of New York, charged a group of three obstetric anesthesiologists and one obstetrician with the proposal of guidelines for safe obstetric anesthesia. The guidelines were finalized in June, 1974, endorsed by the Obstetric Advisory Committee to the Department of Health as "Recommended Standards for Obstetric Anesthetic Care" in August, and implemented in all New York City hospitals rendering obstetric care as of January, 1975. They define standards pertaining to personnel, anesthesia machines and other equipment, drugs, and supplies, including labor, delivery, and recovery rooms. As to personnel, they state that, "Responsibility for obstetric anesthesia should rest with the Director or Chief of Obstetric Anesthesia Service, appointed by the Chairman of the Department of Anesthesiology. The

Director or Chief of Obstetric Anesthesia should be a qualified anesthesiologist. He should be responsible for the technical medical policies and procedures for the conduct of the service and for the supervision of the service. . . . The Director or Chief of Obstetric Anesthesia should establish guidelines approving those who are to administer or to supervise the administration of anesthesia to the obstetric patients. Those approved should be fully competent in all forms of inhalation, intravenous and regional anesthesia as well as in the management of maternal and perinatal complications. At least one such qualified person should be on hospital premises in the vicinity of the delivery suite at all times and should have no other concomitant responsibilities."

The wisdom of advocating the continuous presence of a qualified anesthesiologist in the obstetric suite has been emphasized by three recent emergencies occurring in our delivery rooms. All three developed in patients delivered by the Lamaze method. The first was that of a grand mal seizure with aspiration of regurgitated gastric contents during administration of a pudendal block to a healthy primigravida. A diagnosis of inadvertent intravenous injection of 1 per cent lidocaine was made. The anesthesiologist, called from his nearby office, was able to start treatment within one minute. Therapy consisted of pharyngeal and tracheal suctioning, intravenous injection of 200 mg. of thiopental, and administration of 100 per cent oxygen with intermittent positive pressure via an endotracheal tube. Since oral antacids are given to all our obstetric patients regardless of the planned anesthetic method, the pH of the aspirate was above three, and the patient made an uneventful recovery after she regained consciousness. The infant, delivered by outlet forceps immediately after the convulsion, was in good condition.

The second patient, a healthy 22-year-old woman, was delivered of her second baby spontaneously after local infiltration of the episiotomy site; she was in a semisitting position to facilitate her bearing-down efforts. Shortly following expulsion of the placenta, she complained of feeling faint and appeared pale. The anesthesiologist was summoned. On entering the delivery room, he noticed a "pool" of blood underneath the table which had not been observed by either the obstetrician or the nurse. Quick palpation revealed a fast and thready pulse. The table was immediately leveled, the rate of the intravenous infusion of 5 per cent dextrose in lactated Ringer's solution was increased, and oxygen was administered by face mask. Only then was the blood pressure taken (80/40 mm. Hg). Blood loss was estimated to exceed 1,000 ml., but, aided by a predelivery hematocrit of 38 per cent, the patient recovered without blood replacement. The postpartum hematocrit was 26 per cent.

The third complication involved the newly delivered infant. Just before birth, thick meconium started to extrude from the vagina, and the anesthesiologist was promptly called. The infant, born cyanotic and limp, was immediately placed in the heated crib. Following rapid pharyngeal suctioning, the trachea was intubated and aspirated, yielding a large clot of viscid meconium. The infant's postnatal course was benign, with only mild respiratory distress.

Instead of being "summoned" in case of emergency, the anesthesiologist or anesthetist should be encouraged to participate actively in every delivery. Monitoring of vital functions, diagnosis of the cause of complications, and institution of specific treatment are of the same significance to maternal and neonatal well-being as is the safe administration of anesthesia. Nationwide implementation of the New York City guidelines will assure the best possible care of mother and infant throughout our country.

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Sex selection

To the Editors:

Monarchs have traditionally wanted a boy first as heir to their throne. This has held true for most parents. In response to a questionnaire sent to 4,000 couples selected at random from family planning clinic rosters, approximately two thirds wanted a boy first, with the next child almost invariably desired to be a girl. If two or three of the same sex had occurred, then the opposite was desired. Most would be content with a boy and girl. If one child had been lost, most often one of the same sex was wished for replacement and very seldom were two of the same wanted. Almost identical responses were obtained by Hatzold[5] of Munich, Germany, in 1974.

Influencing the behavior of the sex-determining sperm is not free from controversy. Etzioni[3] has stressed the possible negative and positive social implications of the power to preselect the sex of the offspring. Some have expressed the opinion that this line of research should not be pursued at all, that people should not have this kind of power and choice for fear that passing fads and preferences could drastically overbalance the population one way or the other. Others favor this option as a means of reducing the population, stressing that most parents who might go on trying for a boy or girl would probably be content with one of each.

Information in this field indicates that the day when preconceptional sex selection is possible may not be too far off, in which event education in this area will be very important, just as it is in good health, nutrition, and birth control. Much is already known about aiding

nature in choosing the baby's sex by judging the cervical milieu and timing coitus.[11, 12]

The principle involved is related to the characteristics peculiar to the X and Y sperm. Painter[9] studied the behavior of the sex chromosomes during meiotic cellular division. He found that in both the primary spermatocyte of the first meiotic division and the secondary spermatocyte of the second meiotic division, the X and Y chromosomes and the divided X and Y, respectively, separate early and precede the autosomes to a central position at the opposite poles of the dividing cell. When stained with fluorochrome quinacrine and viewed under fluorescence microscopy, Ericsson and associates[2] found that the smaller-headed sperm contain the Y chromosome.

The modal value on the frequency distribution curve in 500 individuals revealed that 66 per cent were of the smaller-headed type, which have a greater migratory rate when tested in capillary tubes filled with ovulatory cervical mucus.[11] The Millipore filter with appropriate pore size yields a differential filtration of 96 to 98 per cent, i.e., for passage of the Y sperm, into optimal cervical mucus.

Universally, more males are conceived and born; however, in utero and in every age group throughout life more males die.[13] The physical and chemical characteristics of the ovulatory cervical secretion preferentially favor the motility rate of the more prevalent Y sperm and, consequently, male off-spring. Levy[8] and Guttmacher[4] cite the average birth ratio as 100 females/105 to 107 males. The former found that "Among the Jews of Zarist Russia the ratio was 100:150, sometimes even higher." Fifty years ago, he showed that this ratio ". . .only proves right among orthodox Jews, whose conjugal life is conducted according to religious regulations." Accordingly, these couples have their first coitus near or at ovulation time with the cervical milieu optimal for conception.

By controlling the time of artificial insemination or coitus, the birth sex ratios have been significantly altered. The nearer the insemination to ovulation, the greater the incidence of males born. Kleegman,[6, 7] Benedo,[1] and Rorvik and Shettles[10] attained 80 to 85 per cent success rates. In other words, fresh egg and fresh sperm enhance the possibilities for male offspring, while fresh egg and older sperm tend to produce females.

As the world population increases, it becomes more likely that the decision of whether or not to have children will be paramount. It is conceivable that the number of offspring might be limited in order to live within the limits of available food, space, and resources. Many religions and cultures prize a son and encourage a couple to continue until a male is born. If the first or second child could be assured to be male, there would be no need for such large families.

If parents could select the sex of their children and not just settle, they surely would by and large elect to do so, making for greater ease physically, economically, and emotionally. There is the risk of abuse of such information, an argument against sex selection. However, if sex selection is used properly, untold happiness should ensue.

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Reply to Dr. Polansky

To the Editors:

This letter is in reply to Dr. Polansky's Letter to the Editors published in the Journal (122: 903, 1975) concerning my article, "Androgens in breast cancer. III. Breast cancer recurrences years after mastectomy and increased androgenic activity" (Am. J. Obstet. Gynecol. 121: 169, 1975).

Dr. Polansky concluded that estrogens are responsible for breast cancer development, whether the origin of the estrogens is ovarian or peripheral, from androgen conversion.

Estrogens perhaps may be the final substances which act on the breast at the cellular level to initiate abnormal proliferation, but this assumption does not invalidate my theory that increased androgen production initiates the whole mechanism. In breast cancer patients, whether pre- or post-menopausal, the ovarian interstitial tissue may be hyperplastic, and, as this side of the ovary is involved in androgenic activity, it is quite logical to deduce that ovarian androgenic activity will be increased.

If we could inhibit or even depress ovarian androgen production in all women, and especially in postmenopausal women, with abnormal androgen activity, we would perhaps effect a very promising prophylactic correction, even if estrogens were the final product directly involved in breast cancerogenesis, because by inhibiting ovarian androgen activity we would reduce estrogen output at its source.

Dr. Polansky's suggestion that I should make a parallel study of estrogen and androgen behavior in breast cancer patients is something I have done (AM. J. OBSTET. GYNECOL. 116: 423, 1973; 118: 173, 1974).

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BOOKS

Books received

Chemotherapy of Urogenital Tumors. Gerald P. Murphy and Arnold Mittelman. 268 pages, illustrated. Springfield, Illinois, 1975, Charles C Thomas, Publisher. $24.50.

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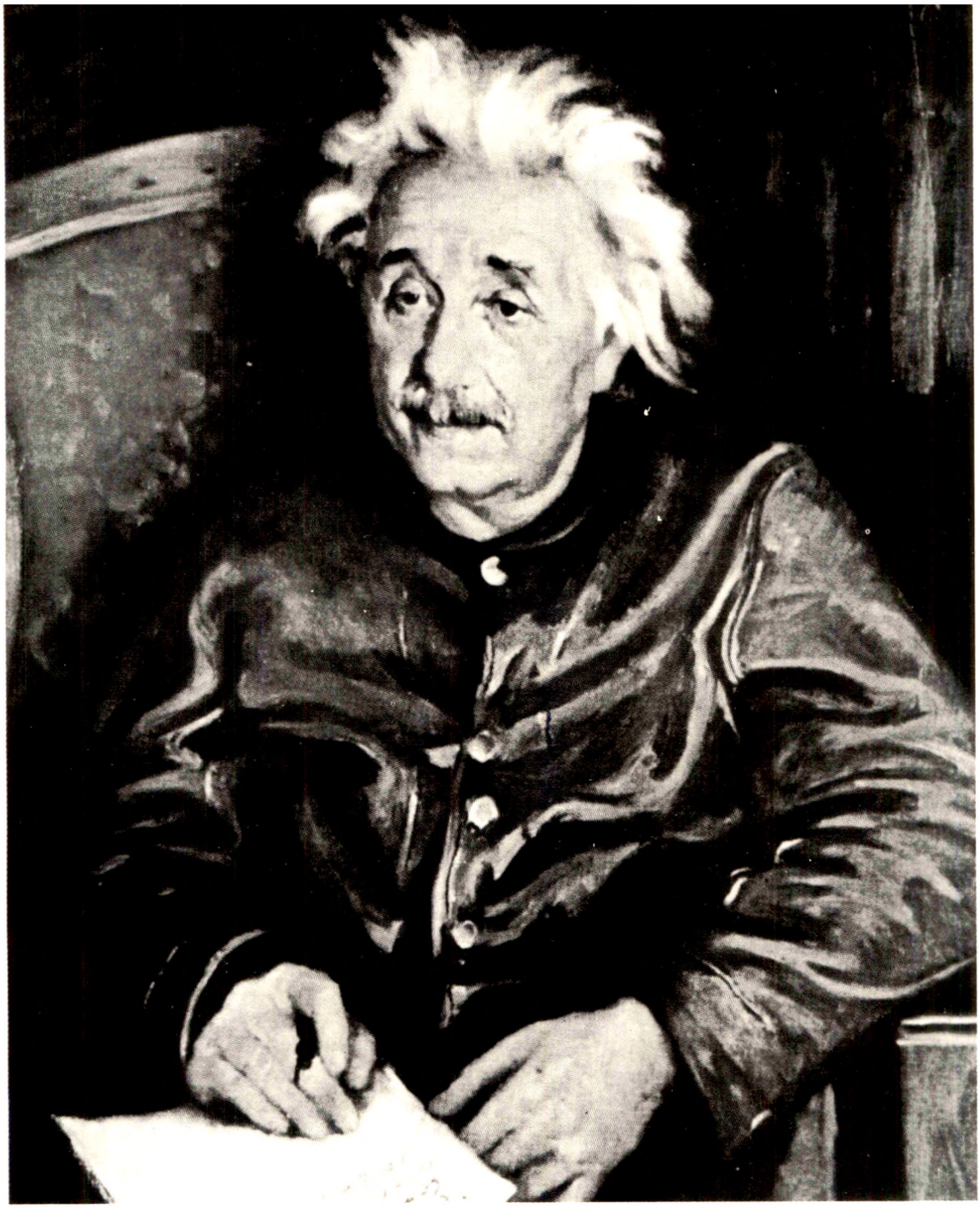
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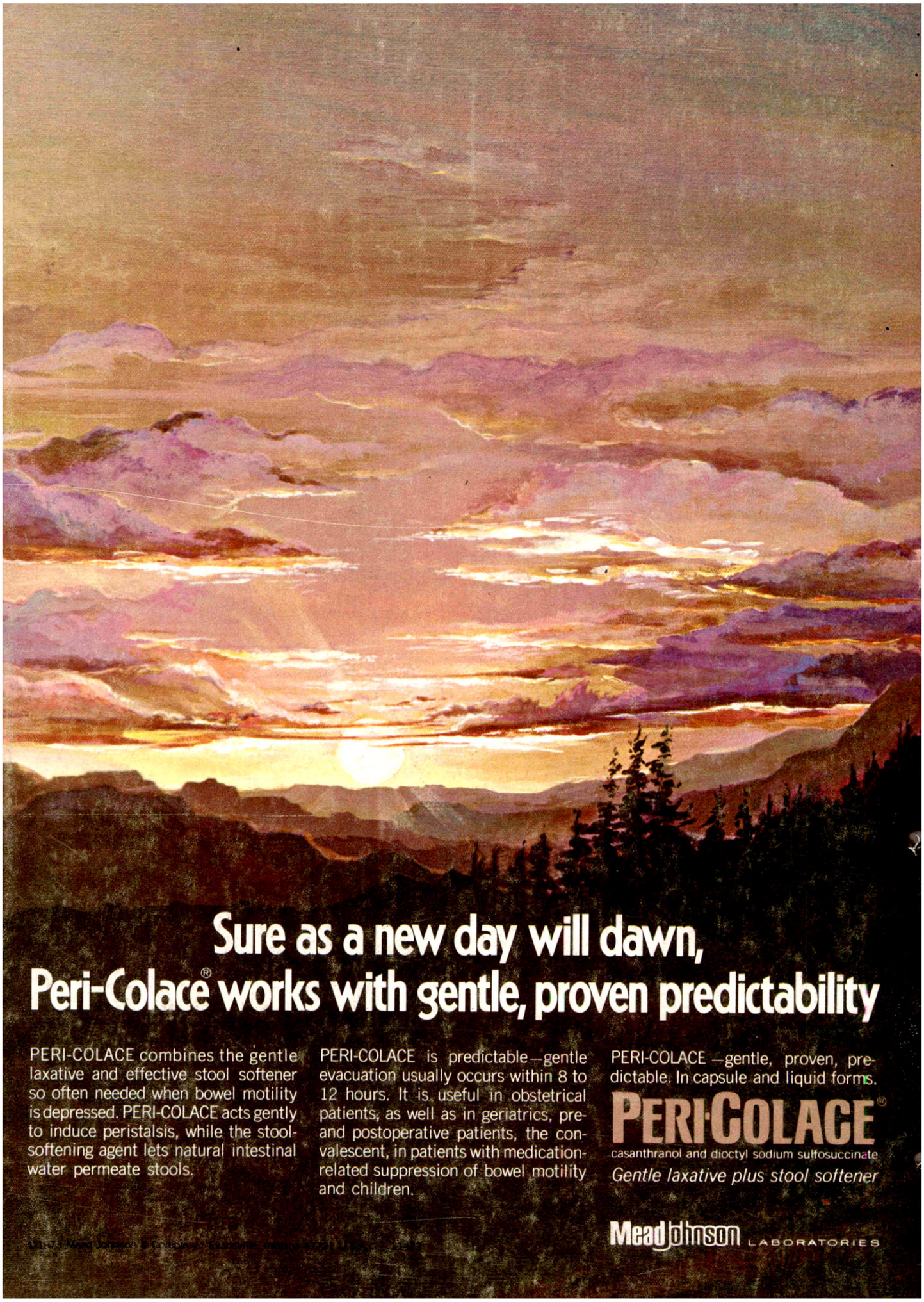
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NORINYL® 1+50

tablets (norethindrone 1 mg with mestranol 0.05 mg)

Special Note

Oral contraceptives have been marketed in the United States since 1960. Reported pregnancy rates vary from product to product. The effectiveness of the sequential products appears to be somewhat lower than that of the combination products. Both types provide almost completely effective contraception.

An increased risk of thromboembolic disease associated with the use of hormonal contraceptives has now been shown in studies conducted in both Great Britain and the United States. Other risks, such as those of elevated blood pressure, liver disease and reduced tolerance to carbohydrates, have not been quantitated with precision.

Long term administration of both natural and synthetic estrogens in subprimate animal species in multiples of the human dose increases the frequency for some animal carcinomas. These data cannot be transposed directly to man. The possible carcinogenicity due to the estrogens can neither be affirmed nor refuted at this time. Close clinical surveillance of all women taking oral contraceptives must be continued.

Indications

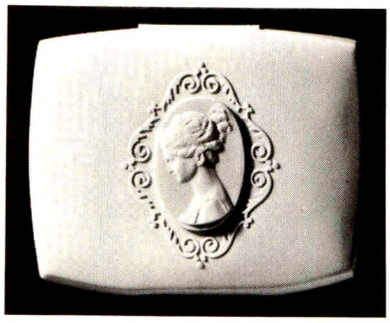
NORINYL 1+50 and NORINYL 1+80 are indicated for oral contraception.

Contraindications

1. Thrombophlebitis, thromboembolic disorders, cerebral apoplexy, or a past history of these conditions.
2. Markedly impaired liver function.
3. Known or suspected carcinoma of the breast.
4. Known or suspected estrogen dependent neoplasia.
5. Undiagnosed abnormal genital bleeding.
6. Known or suspected pregnancy.

Warnings

1. The physician should be alert to the earliest manifestations of thrombotic disorders (thrombophlebitis, cerebrovascular disorders, pulmonary embolism, and retinal thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. Retrospective studies of morbidity and mortality in Great Britain and studies of morbidity in the United States have shown a statistically significant association between thrombophlebitis, pulmonary embolism, and cerebral thrombosis and embolism and use of oral contraceptives. There have been three principal studies in Britain[1,2,3] leading to this conclusion, and one[4] in this country. The estimate of the relative risk of thromboembolism in the study by Vessey and Doll[3] was about sevenfold, while Sartwell and associates in the United States found a relative risk of 4.4, meaning that the users are several times as likely to undergo thromboembolic disease without evident cause as non-users. The American study also indicated that the risk did not persist after discontinuation of administration, and that it was not enhanced by long continued administration. The American study was not designed to evaluate a difference between products. However, the study suggested that there might be an increased risk of thromboembolic disease in users of sequential products. This risk cannot be quantitated, and further studies to confirm this finding are desirable.
2. Discontinue medication pending examination if there is sudden partial or complete loss of vision, or if there is a sudden onset of proptosis, diplopia or migraine. If examination reveals papilledema or retinal vascular lesions, medication should be withdrawn.
3. Since the safety of NORINYL 1+50 and NORINYL 1+80 in pregnancy has not been demonstrated, it is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule the possibility of pregnancy should be considered at the time of the first missed period.
4. A small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The long range effect to the nursing infant cannot be determined at this time.
5. Hepatic cell adenomas have been reported in women on oral contraceptives. The adenoma may present as an abdominal mass or with the signs and symptoms of an acute abdomen. A bleeding hepatic cell adenoma should be considered if the patient has abdominal pain or evidence of intra-abdominal bleeding.



Norethindrone/mestranol 1+50 combination. From the company that developed norethindrone.

Precautions

1. The pretreatment and periodic physical examinations should include special reference to liver, breasts and pelvic organs, including Papanicolaou smear since estrogens have been known to produce tumors, some of them malignant, in five species of subprimate animals.
2. Endocrine and possibly liver function tests may be affected by treatment with NORINYL 1+50 and NORINYL 1+80. Therefore, if such tests are abnormal in a patient taking NORINYL 1+50 and NORINYL 1+80, it is recommended that they be repeated after the drug has been withdrawn for 2 months.
3. Under the influence of estrogen-progestogen preparations, pre-existing uterine fibromyomata may increase in size.
4. Because these agents may cause some degree of fluid retention, conditions which might be influenced by this factor, such as epilepsy, migraine, asthma, cardiac or renal dysfunction, require careful observation.
5. In breakthrough bleeding, and in all cases of irregular bleeding per vaginam, nonfunctional causes should be borne in mind. In undiagnosed bleeding per vaginam, adequate diagnostic measures are indicated.
6. Patients with a history of psychic depression should be carefully observed and the drug discontinued if the depression recurs to a serious degree.
7. Any possible influence of prolonged NORINYL 1+50 and NORINYL 1+80 therapy on pituitary, ovarian, adrenal, hepatic or uterine function awaits further study.
8. A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. The mechanism of this decrease is obscure. For this reason, diabetic patients should be carefully observed while receiving NORINYL 1+50 and NORINYL 1+80 therapy.
9. The age of the patient constitutes no absolute limiting factor, although treatment with NORINYL 1+50 and NORINYL 1+80 may mask the onset of the climacteric.
10. The pathologist should be advised of NORINYL 1+50 and NORINYL 1+80 therapy when relevant specimens are submitted.
11. Susceptible women may experience an increase in blood pressure following administration of contraceptive steroids.

Adverse Reactions Observed in Patients Receiving Oral Contraceptives

A statistically significant association has been demonstrated between use of oral contraceptives and the following serious adverse reactions: thrombophlebitis, pulmonary embolism, cerebral thrombosis.

Although available evidence is suggestive of an association, such a relationship has been neither confirmed nor refuted for the following serious adverse reactions: neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions are known to occur in patients receiving oral contraceptives: nausea, vomiting, gastrointestinal symptoms (such as abdominal cramps and bloating), breakthrough bleeding, spotting, change in menstrual flow, amenorrhea during and after treatment, edema, chloasma or melasma, breast changes: tenderness, enlargement and secretion, change in weight (increase or decrease), changes in cervical erosion and cervical secretions, suppression of lactation when given immediately post-partum, cholestatic jaundice, migraine, rash (allergic), rise in blood pressure in susceptible individuals, mental depression.

Although the following adverse reactions have been reported in users of oral contraceptives, an association has been neither confirmed nor refuted: anovulation post treatment, premenstrual-like syndrome, changes in libido, changes in appetite, cystitis-like syndrome, hirsutism, loss of scalp hair, erythema multiforme, headache, nervousness, dizziness, fatigue, backache, erythema nodosum, hemorrhagic eruption, itching.

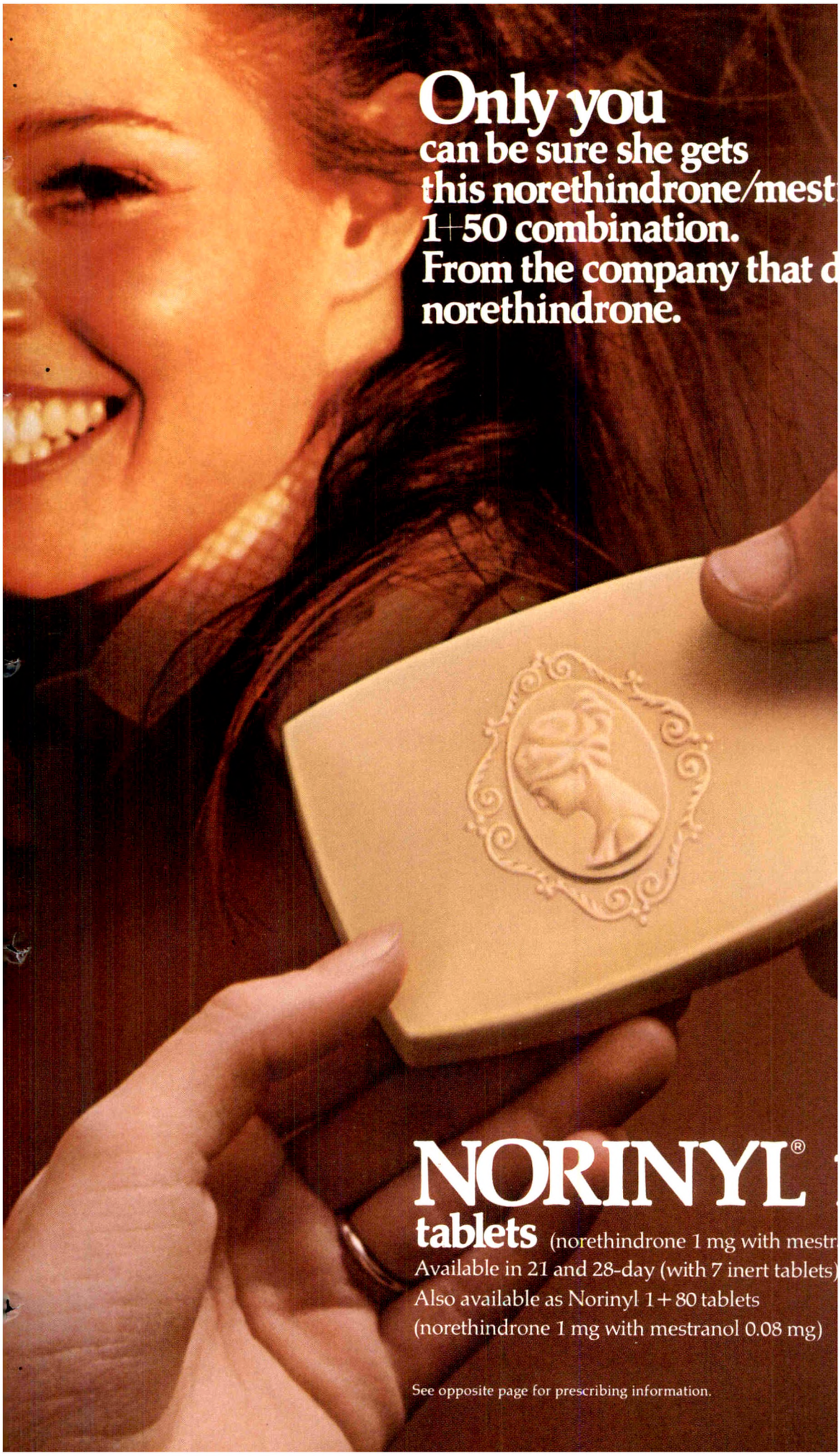
The following laboratory results may be altered by the use of oral contraceptives: hepatic function: increased sulfobromophthalein retention and other tests, coagulation tests: increase in prothrombin factors VII, VIII, IX, and X, thyroid function: increase in PBI, and butanol extractable protein bound iodine and decrease in T^3 uptake values, metyrapone test, pregnanediol determination.

[1]Royal College of General Practitioners: Oral Contraception and Thromboembolic Disease. J. Coll. Gen. Pract., 13:267-279, 1967.
[2]Inman, W.H.W. and Vessey, M.P. Investigation of Deaths from Pulmonary Coronary and Cerebral Thrombosis and Embolism in Women of Child-Bearing Age, Brit Med J, 2:193-199, 1968.
[3]Vessey, M.P. and Doll, R. Investigation of Relation between Use of Oral Contraceptives and Thromboembolic Disease. A Further Report. Brit Med J, 2:651-657, 1969.
[4]Sartwell, P.E., Masi, A.T., Arthes, F.G., Greene, G.R. and Smith, H.E., Thromboembolism and Oral Contraceptives: An Epidemiological Case-Control Study. Am. J.Epidem. 90:365-380, (Nov.) 1969.

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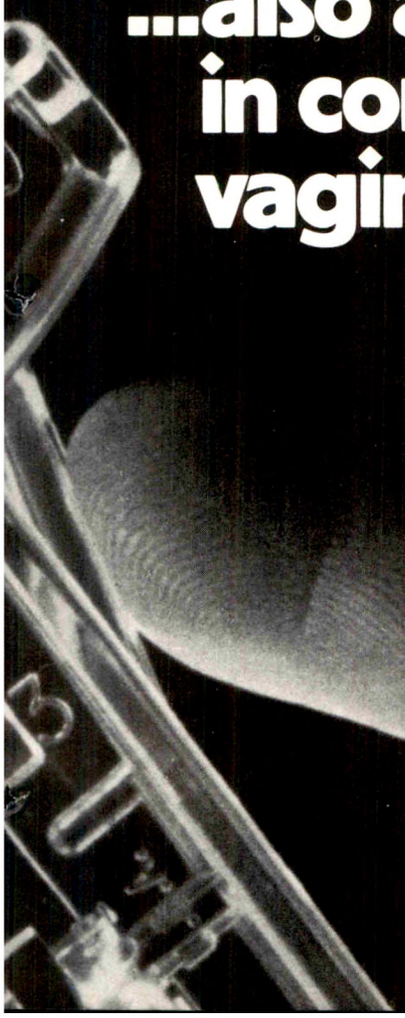
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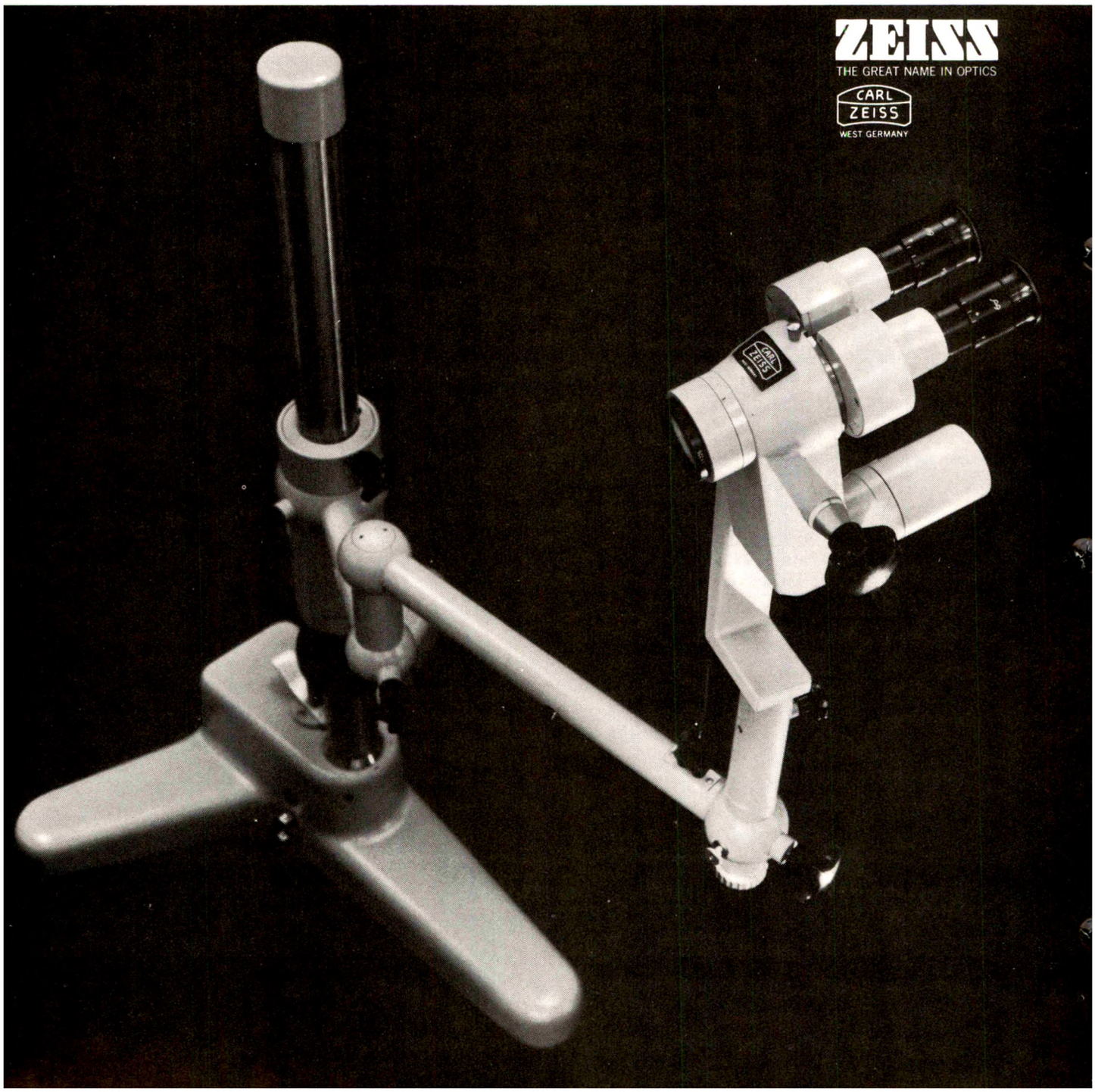
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"Possibly" effective: For the treatment of trichomoniasis, vulvovaginal candidiasis, and vaginitis due to *Hemophilus vaginalis* or other susceptible bacteria.

Final classification of the less-than-effective indications requires further investigation.

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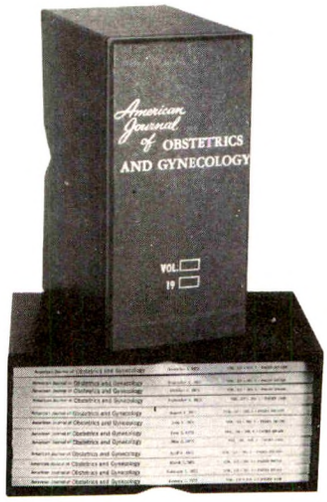
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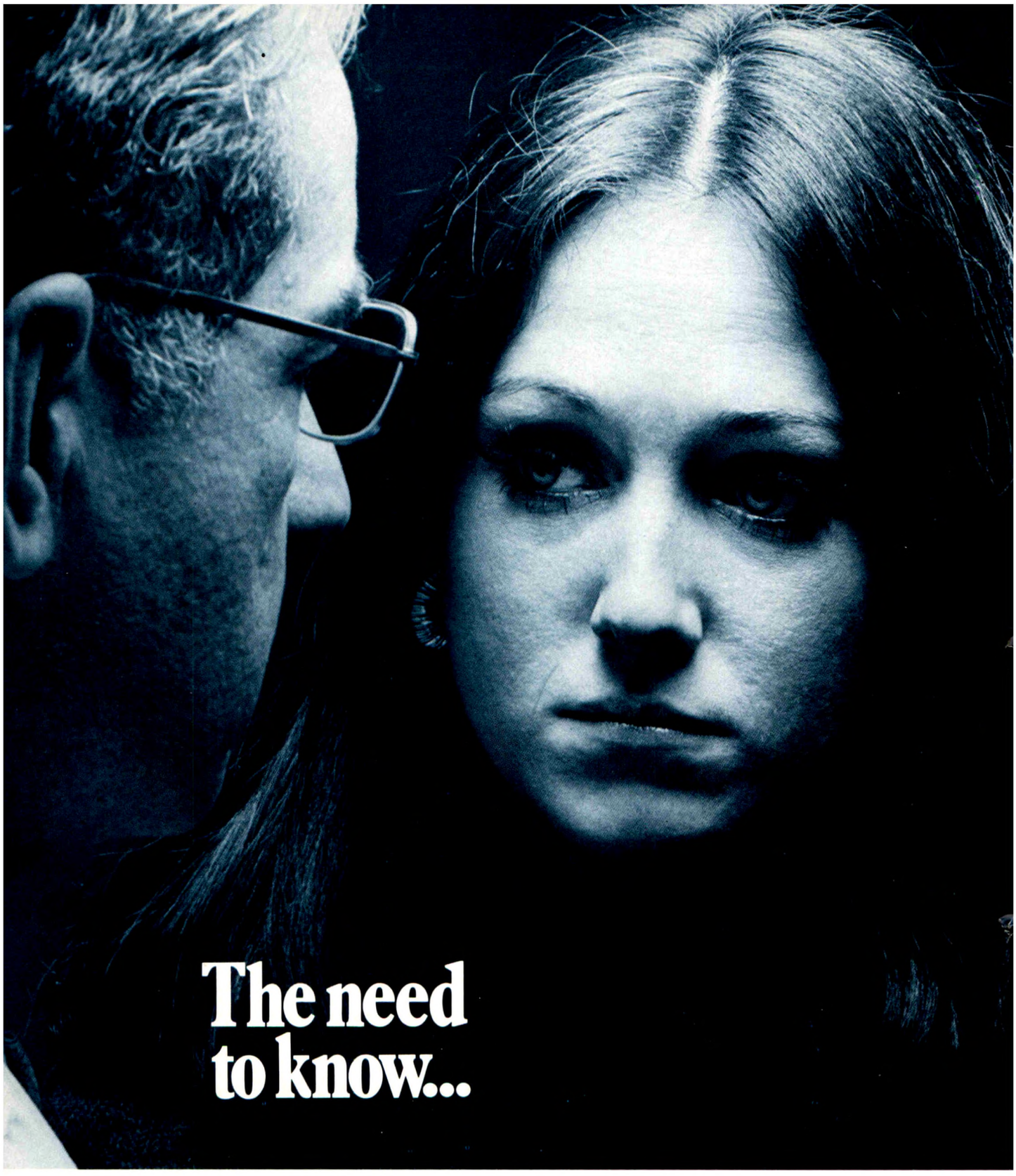
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Breakthrough Bleeding	9.0%	3.5%	2.7%	2.6%	3.9%

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*Serious as well as minor adverse reactions have been reported following the use of all oral contraceptives. The physician should remain alert to their earliest manifestations and in the event of serious adverse reactions should avoid any oral contraceptives. *Special note, contraindications, warnings, precautions and adverse reactions* in the accompanying IN BRIEF should be carefully considered.

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Low dose oral contraceptive.

IN BRIEF:

SPECIAL NOTE: Oral contraceptives have been marketed in the United States since 1960. Reported pregnancy rates vary from product to product. The effectiveness of the sequential products appears to be somewhat lower than that of the combination products. Both types provide almost completely effective contraception.

An increased risk of thrombotic, thromboembolic and cerebral-vascular disease associated with the use of hormonal contraceptives has been shown in studies conducted in both Great Britain and the United States. Other risks, such as those of elevated blood pressure and reduced tolerance to carbohydrates, have been reported, and appropriate tests should be conducted to monitor these during oral-contraceptive therapy. Liver disease has also been reported, and the physician should be alert to its earliest manifestations.

Long-term administration of both natural and synthetic estrogens in subprimate animal species in multiples of the human dose increases the frequency for some animal carcinomas. These data cannot be transposed directly to man. The possible carcinogenicity due to the estrogens can neither be affirmed nor refuted at this time. Close clinical surveillance of all women taking oral contraceptives must be continued.

INDICATIONS: For oral contraception.

CONTRAINDICATIONS: 1. Thrombophlebitis, thromboembolic disorders, cerebral-vascular disease, or a past history of these conditions; 2. Markedly impaired liver function; 3. Known or suspected carcinoma of the breast; 4. Known or suspected estrogen-dependent neoplasia; 5. Undiagnosed abnormal genital bleeding; 6. Known or suspected pregnancy.

WARNINGS: 1. The physician should be alert to the earliest manifestations of thrombotic and thromboembolic disorders, thrombophlebitis, cerebrovascular disorders including hemorrhage, pulmonary embolism and retinal thrombosis. Should any of these occur or be suspected, the drug should be discontinued immediately.

Retrospective studies of morbidity and mortality in Great Britain and studies of morbidity in the United States have shown a statistically significant association between thrombophlebitis, pulmonary embolism and cerebral-vascular disease, occlusive or hemorrhagic, and the use of oral contraceptives. There have been three principal studies in Great Britain[1-3] leading to these conclusions and three[4-7] in this country. The estimate of the relative risk of thromboembolism in the study by Vessey and Doll[3] was about sevenfold, while the United States studies found relative risks of 4.4 to 11, meaning that the users are several times as likely to undergo thromboembolic disease without evident cause as non-users.

In May, 1974, the Royal College of General Practitioners in England[8] issued an interim report of its continuing large-scale prospective study comparing a user group to a non-user group. This study in its interim analysis states: "A statistically significant higher rate of reporting of cerebrovascular accidents in Takers is evident, but the numbers are too small to justify an estimation of the degree of risk." The study also reported a higher incidence of superficial and deep vein thrombosis in users as compared to non-users.

The Sartwell study[4] indicated that the risk did not persist after discontinuation of administration. Both the Sartwell and the Royal College study indicated that the degree of risk was not associated with duration of treatment.

In a collaborative American study[5,6] of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the relative risk of thrombotic stroke was 4.1 to 9.5 times greater in users than in non-users. A comparable estimate for hemorrhagic stroke was 2.0.

None of the American studies was designed to evaluate a difference between products. However, the Sartwell study[4] suggested that there might be an increased risk of thromboembolic disease in users of sequential products.

Other retrospective studies[9,10] have reported an increased risk of post-surgery thromboembolic complications in oral-contraceptive users. It has been recommended that therapy be discontinued at least one month prior to elective surgery.

2. Discontinue oral-contraceptive medication if there is: gradual or sudden, partial or complete loss of vision; proptosis or diplopia; onset or aggravation of migraine or development of headache of a new pattern which is recurrent, persistent or severe; papilledema; or any evidence of retinal-vascular lesions. 3. Fetal abnormalities have been reported to occur in the offspring of women who have taken progestogens and/or estrogens during pregnancy.[11,12] The safety of LO/OVRAL® in pregnancy has not been demonstrated. Pregnancy should be ruled out before initiating or continuing the contraceptive regimen. Pregnancy should always be considered if withdrawal bleeding does not occur. 4. A small fraction of the hormonal agent in oral contraceptives has been identified in the milk of mothers receiving these drugs. The long-range effect to the nursing infant cannot be determined at this time. 5. Hepatic lesions (adenomas, hepatomas, hamartomas, regenerating nodules, etc.), occasionally fatal, have been reported in women on oral contraceptives. Such lesions may present as an abdominal mass or with the signs and symptoms of an acute abdomen. These lesions should be considered if the patient has abdominal pain or evidence of intra-abdominal bleeding. Such lesions have been reported in short-term as well as long-term users of oral contraceptives.

PRECAUTIONS: 1. A thorough history and physical examination should be performed before prescribing oral contraceptives and periodically during their administration and should include special reference to breasts and pelvic organs including Papanicolaou smears, since estrogens have been known to produce tumors, some of them malignant, in five species of subprimate animals. 2. Endocrine and possibly liver-function tests may be affected by treatment with LO/OVRAL® Therefore, if such tests are abnormal in a patient taking LO/OVRAL® it is recommended that they be repeated after the drug has been withdrawn for two months. 3. Under the influence of estrogen-progestogen preparations, pre-existing uterine fibromyomata may increase in size. 4. Because these agents may cause some degree of fluid retention, conditions which might be influenced by this factor, such as epilepsy, migraine, asthma, cardiac or renal dysfunction, require careful observation. 5. In breakthrough bleeding, and in all cases of irregular bleeding per vaginam, nonfunctional causes should be borne in mind. In undiagnosed bleeding per vaginam, adequate diagnostic measures are indicated. 6. Patients with a history of psychic depression should be carefully observed and the drug discontinued if the depression recurs to a serious degree. 7. Any possible influence of prolonged LO/OVRAL® therapy on pituitary, ovarian, adrenal, hepatic or uterine function awaits further study. 8. A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. The mechanism of this decrease is obscure. For this reason, diabetic patients should be carefully observed while receiving LO/OVRAL® therapy. 9. The age of the patient constitutes no absolute limiting factor, although treatment with LO/OVRAL® may mask the onset of the climacteric. 10. The pathologist should be advised of LO/OVRAL® therapy when relevant specimens are submitted. 11. Susceptible women may experience an increase in blood pressure following administration of contraceptive steroids. 12. Cholestatic jaundice has been reported in users of oral contraceptives. If this occurs, LO/OVRAL® should be discontinued. This condition is more likely to occur in patients who have experienced cholestatic jaundice of pregnancy. Patients with a history of cholestatic jaundice of pregnancy should be carefully observed during LO/OVRAL® therapy.

ADVERSE REACTIONS OBSERVED IN PATIENTS RECEIVING ORAL CONTRACEPTIVES: A statistically significant association has been demonstrated between use of oral contraceptives and the following serious adverse reactions: thrombophlebitis, pulmonary embolism, cerebral thrombosis and hemorrhage, gall bladder disease.

Although available evidence is suggestive of an association, such a relationship has been neither confirmed nor refuted for the following serious adverse reactions: neuro-ocular lesions, e.g., retinal thrombosis, optic neuritis and hepatic lesions with or without intra-abdominal bleeding.

The following adverse reactions are known to occur in patients receiving oral contraceptives: nausea, vomiting, gastrointestinal symptoms (such as abdominal cramps and bloating), breakthrough bleeding, spotting, change in menstrual flow, amenorrhea during and after treatment, edema, chloasma or melasma, which may persist, breast changes: tenderness, enlargement and secretion, change in weight (increase or decrease), changes in cervical erosion and cervical secretions, suppression of lactation when given immediately post-partum, cholestatic jaundice, migraine, rash (allergic), rise in blood pressure in susceptible individuals, mental depression.

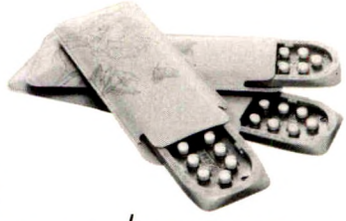
Although the following adverse reactions have been reported in users of oral contraceptives, an association has been neither confirmed nor refuted: anovulation post treatment, which tends to occur more frequently in women with a history of menstrual disorders, premenstrual-like syndrome, changes in libido, changes in appetite, cystitis-like syndrome, headache, intolerance to contact lenses, nervousness, dizziness, fatigue, backache, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, itching and vaginitis.

The following laboratory results may be altered by the use of oral contraceptives: hepatic function; increased sulfobromophthalein retention and other tests; coagulation tests; increase in prothrombin, Factors VII, VIII, IX and X; decrease in antithrombin III; increase in platelet aggregability; thyroid function: increase in PBI, and butanol extractable protein-bound iodine and decrease in T^3 uptake values; metyrapone test; and pregnanediol determination.

NOTE: For more specific details on LO/OVRAL, refer to package insert.

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EDITORIAL

Summary of analysis of published articles

September 1, 1974, through August 31, 1975

THE EDITORS present a retrospective analysis of the category and type of articles published during the past two years, with a 12 month period for each year (Table I). These data are gathered to keep the Editorial Staff informed about the kinds of articles received from authors and published. We share this information with the readers because we believe it to be of value and of interest to them, as well.

Table I. Analysis of published articles

Category	*1974*		*1975*	
	No. of articles	*Pages*	*No. of articles*	*Pages*
Journal division				
Obstetrics	187	1,201	193	1,141
Gynecology	217	1,269	221	1,336
Fetus, placenta, and newborn	112	703	90	536
Education	20	135	11	88
Total	536	3,308	515	3,101
Type of article				
Clinical	196	1,066	198	1,092
Clinical investigation	207	1,358	199	1,225
Basic research	133	884	118	784
Total	536	3,308	515	3,101
Average length of articles		6.7		6.0

OBSTETRICS

The remote prognosis of eclamptic women

Sixth periodic report

LEON C. CHESLEY, PH.D.*
JOHN E. ANNITTO, M.D.
ROBERT A. COSGROVE, M.D.
Jersey City, New Jersey

All but three of the 270 women surviving eclampsia at the Margaret Hague Maternity Hospital in the period 1931 through 1951 were traced to 1973-74. Seventy-six have died and 13 were not re-examined. In white women having eclampsia in the first pregnancy carried to viability the remote mortality rate is not increased over that in unselected women; in white women having eclampsia as multiparas and in all black women the remote mortality rate is from 2 to 5 times the expected numbers. Primiparous eclamptic women are not different from women matched for age, in several epidemiologic studies, in the prevalence of hypertension or in the frequency distributions of systolic and diastolic blood pressures. There is, however, a considerable increase in the prevalence of hypertension among women having had eclampsia as multiparas and that has accounted for their increased remote death rates. The prevalence of diabetes, developing many years after eclampsia, is 2.5 times the expected rate in primiparous and about 4 times the expected rate among multiparous eclamptic women. Eclampsia neither is a sign of latent essential hypertension nor causes hypertension. Hypertensive pregnancies following eclampsia indicate the probability of later chronic hypertension, but do not cause it.

FOLLOW-UP studies of women having had eclampsia were begun in Germany a century ago, when eclampsia was widely believed to be a form of uremia. The questions were whether the women had acute or chronic renal disease and, if eclampsia represented acute nephritis, whether prolongation of the pregnancy would aggravate the renal lesion, prevent healing, and result in chronic impairment. Frerichs[14] concluded that eclampsia sometimes gives rise to chronic nephritis and Schroeder[39] wrote emphatically that unless eclamptic pregnancies were terminated without delay, chronic nephritis would ensue. An alternative hypothesis of that time, expressed by Spiegelberg,[40] was that eclampsia resulted from severe renal vasospasm reflexly evoked by uterine distention and that, if the vasoconstriction were maintained long enough, it might cause irreversible renal disease.

The findings in the early follow-up studies were indeterminate, by modern standards, and it was not until 60 years ago that Wolff and Zade[45] of Heidelberg

From the Margaret Hague Maternity Hospital.

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Table I. Chronic hypertension following eclampsia: A summary of the literature

Author(s)	*Date*	*City*	*Orig. No. of cases*	*Immed. survivors*	*Remote deaths*	*Re-examined*	*Length of follow-up*	*Hypertension**
Chesley and Somers,[10] review of 41 papers	1941		?	?	?	1,454	6 wk.-28 yr.	290†
McClellan et al.[30]	1942	Nashville	75	65	1	30	6 mo.-14 yr.	12
Quirno et al.[35]	1943	Buenos Aires	?	220	?	19	3 yr.-16 yr.	4
Kotásek[24]	1948	Prague	?	?	?	51	1 yr.-20 yr.	10
Mastboom[27]	1949	Amsterdam	110	102	6	96	1 yr.-10 yr.	24
Bryans and Torpin[5]	1949	Augusta, Ga.	483	?	27	243	1 yr.-28 yr.	51
Rasmussen et al.[36]	1951	Oslo	?	130	7	111	10 yr.-18 yr.	41
Schreier et al.[38]	1955	Memphis	?	?	?	26	5 yr.-10 yr.	1
Bryans[4]	1966	Augusta, Ga.	?	?	?	335	1 yr.-44 yr.	110
Kremling et al.[25]	1967	Wuerzburg	?	?	?	26	3 mo.-19 yr.	4
Novikova[32]	1967	Moscow (?)	?	?	?	102	2 yr.-7 yr.	41
Thornton[42]	1967	Charlottesville	168	160	?	125	6 wk.-24 yr.	27
Fritzsch et al.[15]	1970	Berlin	30	29	1	19	1 yr.-8 yr.	12
Totals						2,637	6 wk.-44 yr.	627 (23.8%)

*What the earlier investigators called "chronic nephritis" is tabulated as "hypertension"
†0-78 per cent; average 19.9 per cent.

reported measurements of blood pressure; they found hypertension in two of 23 women examined at from 1 to 5 years after eclampsia. At that time, and for many years thereafter, hypertension in young women was taken as a sign of "chronic nephritis," largely because of the lingering implication of the term "senile plethora" that Allbutt[1] had used in differentiating primary from secondary hypertension.

Table I summarizes many of the follow-up studies of women who had had eclampsia; the prevalence of chronic hypertension ranges from 0 to 78 per cent and averages 23.8 per cent in the 2,637 women included in 53 publications. Several factors account for the enormous variation. One of the most important is that in nearly all studies only a few of the original cases were traced and re-examined; the women traced often constituted a highly biased sample. In large medical centers, whence most studies came, the women had eclampsia on the obstetric service; the ones easily traced were those admitted to other services years later, often with an unwitting selection of women admitted to medical services because of hypertensive complications. Similarly, the remote mortality rates have been overestimated by relating the deaths, which are easily traced, to the numbers of women found at follow-up rather than to the original numbers having had eclampsia. Other sources of variation in the reported prevalences of hypertension include the length of follow-up (from 6 weeks to 44 years, in Table I), the ages of the women when re-examined (from the second to the ninth decades of life), variable proportions of multiparas whose eclampsia had been superimposed upon antecedent chronic hypertension, racial factors, and others.

When we began our follow-up studies, nearly 40 years ago, it seemed to us that we could eventually answer some important questions. Fishberg,[13] in the fourth edition of *Hypertension and Nephritis,* expressed a current opinion in writing that pre-eclampsia–eclampsia is not an entity but rather is a sign of latent hypertensive disease brought to light and peculiarly colored by pregnancy. Herrick and Tillman[19] seem to have been of the same opinion. If that be so, long-term follow-up study should find chronic hypertension in nearly all such women who live long enough. Two papers by Peckham[33, 34] were suggestive in that regard. He examined 73 women at 13 months after eclampsia and re-examined 61 of them about 6 years later. He found that the prevalence of "chronic nephritis" had increased from 23 to 38 per cent. Would a continuing follow-up have found more and more to be hypertensive? Heynemann,[20] on the other hand, found that after eclampsia some women with hypertension became normotensive as time went on, which he attributed to healing of residual lesions.

Another popular hypothesis has been that prolonged pre-eclampsia–eclampsia causes vascular damage, resulting in chronic hypertension that some women would otherwise never have developed. If that be true, the prevalence of hypertension at follow-up should be greater than in unselected women matched for age and race.

Another wavelength in the spectrum of hypotheses is that pre-eclampsia–eclampsia is unrelated to chronic hypertension. If that be so, the prevalences of hypertension at follow-up should not be different in posteclamptic and matched control women.

Interpretations have been clouded by studies of women thought to have had pre-eclampsia. In general, the prevalence of chronic hypertension in such women has been found to be about twice that in women who had had eclampsia and to be greater following mild than severe pre-eclampsia. Two explanations have been offered. One is that eclampsia sometimes has an acute onset and that pregnancy is terminated, spontaneously or artificially, after only a short period of hypertension. In contrast, pre-eclamptic pregnancies may go on for weeks and leave residual vascular damage. Although eclampsia occasionally does have an explosive onset, analysis of our data leads us to agree with Bryans,[4] who wrote that eclampsia typically "is the end result of long-standing, neglected, and gradually progressing pre-eclampsia." We, therefore, reject the first explanation. The second explanation is that the difficulties of differential diagnosis result in the inclusion of cases of latent and frank essential hypertension or renal disease that are mistakenly regarded as pre-eclampsia.

There are many indications that the clinical diagnosis of pre-eclampsia, especially mild pre-eclampsia, is often erroneous. The best single evidence comes from electron microscopic examinations of renal biopsies. McCartney[29] carefully selected 62 primigravidas who had been clinically normal until the onset of hypertension, proteinuria, and edema in the third trimester. The clinical diagnosis had to be pre-eclampsia. Only 71 per cent (44 cases) had the renal lesion of pre-eclampsia, which might mean that the lesion is not invariably present. Sixteen (26 per cent), however, had lesions of chronic renal disease; 13 had chronic glomerulonephritis that had been silent during much of pregnancy. Three had no renal lesions at all and may have been cases of latent essential hypertension. If the diagnostic error approached 30 per cent in primigravidas so rigidly selected, what must it be in all cases of gestational hypertension? Moreover, most of the follow-up studies of "pre-eclampsia" have included large proportions of multiparas, in whom the diagnosis is usually erroneous. McCartney selected 152 multiparas on the basis of their having chronic hypertension with apparently superimposed pre-eclampsia. Only 21 (14 per cent) had the renal lesions of pre-eclampsia and if the definitive diagnosis depends upon the anatomic change, the clinical diagnostic error would be 86 per cent. McCartney's findings have been confirmed by Guilhem and co-workers,[17] Janisch,[21] and Thaler,[41] among others.

Our own[11] follow-up study of women thought to have had pre-eclampsia led us to the erroneous conclusion, later retracted,[7] that prolonged pre-eclampsia causes chronic hypertension.

We have re-examined, at intervals of 6 or 7 years, nearly all of the surviving women who have had eclampsia at the Margaret Hague Maternity Hospital in the period from the opening of the institution in 1931 through 1951. Although the diagnosis of eclampsia is not always correct, it is far more secure than the diagnosis of pre-eclampsia. Eclampsia (and "true" pre-eclampsia) is predominantly a disease of the first pregnancy carried to viability and when it occurs in a multipara there is usually a predisposing factor, such as antecedent chronic hypertension. For that reason, women having eclampsia as multiparas should not be used in looking for an effect of eclampsia upon later blood pressures. The present paper is the sixth periodic report of our long-term follow-up study, which now averages 33 years and ranges from 22 to 42 years for the women still alive.

Material and methods

The Margaret Hague Maternity Hospital opened on October 15, 1931; from then through 1951 there were 135,973 deliveries therein, and 300 cases of convulsive eclampsia occurred in 296 women. The 27 immediate deaths gave a maternal case mortality rate of 9 per cent. One black woman survived her first eclampsia, which she had as a multipara, but died of her second eclampsia in a later pregnancy. Thus, 270 women were discharged alive after eclampsia. In 1973-74 we traced all but three, having lost one since 1966; 76 have died and we have proved the survival of all others, except the three who are lost. We have re-examined 178; the other 13, most of whom live in distant places, either have refused or are still procrastinating. At least three of them have visited physicians who refuse to fill out our form. Strangely enough, we have been able to re-examine six of the nine who refused in 1966. The material is summarized in Table II.

The women living nearby were asked to come into the Margaret Hague Clinic for examination; those who did not were examined in their homes. Sixty-two women who live far from the hospital were sent forms for their local physicians to fill out and we paid the bills.

The examination consisted of a measurement of blood pressure with the patient seated, estimation of the resistance of the radial arteries to digital compression, clinical estimation of cardiac size and sounds, often an evaluation of the eyegrounds, and urinalysis.

Table II. Follow-up of women surviving eclampsia

Patients	*Time of eclampsia*				*Totals**
	1931-34	*1935-39*	*1940-45*	*1946-51*	
Discharged alive	76	83	64	50	270
Remote deaths	23	35	14	5	76
Re-examined in 1973	48	46	42	44	178
Refused examination	5	1	6	1	13
Lost	0	1	2	0	3

*Corrected for duplications arising from four women who had eclampsia twice, one of whom appears as a remote death and two of whom were re-examined. The other survived her first eclampsia but died of the second.

Table III. Remote deaths in women surviving eclampsia

	White primiparas	*White multiparas*	*Black primiparas*	*Black multiparas*
No. surviving eclampsia	187	59	19	5
No. traced to 1973-74	185	59	18	5
Remote deaths:				
Number	31	33	7	5
Percentage	16.6	56	36.8	100
Deaths in cardiovascular field:				
Number	9	27	4	3*
Percentage of deaths	29	82	57	60
Patient-years of follow-up	6,067	1,554	508.5	109
Average annual death rate per 1,000	5.11	21.3	13.8	45.8
Expected deaths	25.7	11.6	2.8	0.8
Ratio of actual/expected deaths	1.19	2.75	2.3	5.0
P	0.4†	0.001†	0.03‡	0.004‡
Mean years of follow-up, all cases	32.5	26.3	26.8	21.8
Mean years of follow-up of those still alive	33.5	31.9	32.3	—

*The other two had severe longstanding hypertension, one with diabetes, but they died of malignant tumors.

†P calculated from chi square.

‡P calculated from the Poisson distribution.

The diastolic pressure was recorded as the point at which the pulse sounds suddenly became dull and muffled (phase IV), for that was the recommendation of the American Heart Association in the early days of our study and now is again, after an interim in which disappearance of sounds (phase V) was recommended.

The 64 women having eclampsia in the second or later pregnancies carried to viability are analyzed separately because their remote prognosis is significantly different from that for women having eclampsia in the first pregnancy carried to viability. In the latter group are 18 women who had had early abortions prior to eclampsia; they have been combined with the 188 primigravidas to give 206 of what we shall call "primiparous eclamptic women."

Results

Remote deaths. At the time of our previous follow-up study, in 1966, 44 of the 270 women had died since discharge from the hospital. Since 1966, 32 more deaths have occurred to give a total of 76. Table III summarizes the findings and Table IV shows the causes of death.

White primiparous eclamptic women. A total of 187 survived eclampsia and all but two have been traced to 1973-74; one has had no follow-up at all and one, last seen in 1966, was followed for 26½ years. In all, 31 have died, a remote mortality rate of 16.6 per cent. Only nine (29 per cent) of the deaths were in the cardiovascular field. The average annual death rate over 32.5 years has been 5.11 per 1,000.

We were impressed by the number (12) dying of various malignant tumors and, for that reason, we have compared the actual number with the number to be expected. Burbank[6] has published age-specific death rates from all malignancies in women. We have counted the total patient years lived in each age interval, such as ages 50 to 54, between the ages 15 and 84; multiplying the patient-years by the appropriate age-specific death rate gives the expected number of deaths for each interval of age. Adding those gives the total number of expected deaths, which is eight (7.727

Table IV. Causes of remote deaths in women surviving eclampsia

	White primiparas	*White multiparas*	*Black primiparas*	*Black multiparas*	*Totals*
Cardiovascular field:					
Cerebral hemorrhage	1	13	3	2	19
Myocardial infarction	5	6	0	0	11
Cardiac failure	1	7	0	0	8
Renal failure	2	1	1	0	4
Later eclampsia	0	0	0	1	1
Other causes:					
Malignant tumors	12	3	1	2	18
Tuberculosis	0	1	1	0	2
Pneumonia	2	0	1	0	3
Accident	1	1	0	0	2
Alcoholism	1	0	0	0	1
Septic abortion	1	0	0	0	1
Obstetric hemorrhage	1	0	0	0	1
Hepatitis	1	0	0	0	1
Infection	0	1	0	0	1
Uncertain	3	0	0	0	3
Totals	31	33	7	5	76

is the exact figure derived). The observed number of deaths (12) is not statistically significantly different from the expected eight (p = 0.1, from the Poisson distribution).

White previously parous eclamptic women. A total of 59 women survived eclampsia; all have been traced to 1973-74. Thirty-three have died, a remote mortality rate of 56 per cent. Tweny-seven (82 per cent) of the deaths have been in the cardiovascular field. Three died of malignant tumors, which is exactly the expected number; two others also had malignancies but died of other causes. The average annual death rate over 26.3 years has been 21.3 per 1,000, which is four times that in white primiparous eclamptic women.

Black women. Ninteen black women having eclampsia as primiparas survived the disease. All but one, who was followed for 2½ years and was last seen in 1941, have been traced to 1973-74. Seven have died, a mortality rate of 36.8 per cent. Four of the seven deaths were in the cardiovascular field. The average annual death rate over 26.8 years was 13.8 per 1,000, which is more than twice that in white primiparous eclamptic women.

Five previously parous black women survived eclampsia and all have died at an average age of 49 (range 35 to 63). The average interval from eclampsia to death was 21.8 years and ranged from 5 to 33. All were severely hypertensive, which resulted in the deaths of three; the other two died of malignant tumors. The average annual death rate over 21.8 years was 45.8 per 1,000 which is about four times that in black primiparous eclamptic women and nine times that in white primiparas. In either race the average remote annual death rate is four times greater in multiparous than in primiparous eclamptic women of the same race. The averages are crude death rates, unadjusted for age. Their significance is assessed in the comparison of actual with expected deaths, shown in Table III.

Expected vs. actual remote deaths. Having traced 98.9 per cent of the women surviving eclampsia, we can analyze their survival in relation to normal expectancy. In previous reports we have used data supplied by Mr. Herbert Marks, formerly of the Metropolitan Life Insurance Company. He provided us with age-specific death rates, by race, for each of the calendar years 1931 through 1964, for virtually unselected urban women who paid weekly premiums for life insurance in the Industrial Department of the Company. That form of insurance has been discontinued and for data since 1964 we have used the age-specific death rates for all American women.[31] Comparison of the national death rates with those among the women holding industrial insurance shows a close correspondence in the years for which data are available from both sources.

In calculating the numbers of expected deaths, each annual total of patient-years in each age category, such as ages 45 to 54, was multiplied by the appropriate age-specific death rate to get the "expected" deaths for each year. The expected deaths were then added together to get the data in Table III.

As may be seen in Table III, the white primiparous eclamptic women have not sustained a significant increase in remote deaths thus far in the follow-up. In contrast, the black primiparas and both white and black

Table V. Life table for 187 white and 19 black women who had eclampsia in the first pregnancy carried to viability

Follow-up (yr.)	*No. alive at beginning of year*	*Reason withdrawn*		*Estimated proportion*		*Cumulative surv. (%)*
		End of follow-up	*Death*	*Surviving*	*Dying*	
1	206	1*	3	0.985	0.015	98.5
2	202	0	1	0.995	0.005	98.0
3	201	1*	0	1.000	0.000	98.0
4	200	0	0	1.000	0.000	98.0
5	200	0	0	1.000	0.000	98.0
6	200	0	0	1.000	0.000	98.0
7	200	0	0	1.000	0.000	98.0
8	200	0	2	0.990	0.010	97.0
9	198	0	0	1.000	0.000	97.0
10	198	0	0	1.000	0.000	97.0
11	198	0	1	0.995	0.005	96.5
12	197	0	0	1.000	0.000	96.5
13	197	0	0	1.000	0.000	96.5
14	197	0	0	1.000	0.000	96.5
15	197	0	2	0.987	0.013	95.2
16	195	0	0	1.000	0.000	95.2
17	195	0	1	0.995	0.005	94.7
18	194	0	0	1.000	0.000	94.7
19	194	0	3	0.985	0.015	93.2
20	191	0	1	0.995	0.005	92.6
21	190	0	0	1.000	0.000	92.6
22	190	0	0	1.000	0.000	92.6
23	190	7	2	0.989	0.011	91.4
24	181	6	1	0.994	0.006	90.8
25	174	3	0	1.000	0.000	90.8
26	171	1	3	0.982	0.018	89.2
27	167	11 (1*)	0	1.000	0.000	89.2
28	156	7	1	0.993	0.007	88.5
29	148	6	1	0.993	0.007	87.8
30	141	5	0	1.000	0.000	87.8
31	136	9	0	1.000	0.000	87.8
32	127	12	0	1.000	0.000	87.8
33	115	8	0	1.000	0.000	87.8
34	107	5	2	0.981	0.019	86.1
35	101	10	2	0.979	0.021	84.3
36	89	10	1	0.988	0.012	83.3
37	78	7	2	0.973	0.027	81.1
38	69	11	3	0.953	0.047	77.3
39	55	3	2	0.963	0.037	74.5
40	50	12	2	0.955	0.045	71.1
41	36	8	2	0.937	0.063	66.6
42	26	23	0	1.000	0.000	66.6
43	3	3	0	1.000	0.000	66.6

*Lost to follow-up.

multiparas have suffered a significantly increased remote mortality rate. The excess of deaths is undoubtedly attributable to the high prevalence of hypertensive disease among black women and in the white women who had eclampsia as multiparas.

Life tables. Tables V and VI show many details of the study, such as numbers of women followed year by year and when they died, were lost, or came to the end of the follow-up in 1973-74. White and black women have been combined because of the small numbers of the latter. The last column in each table shows the cumulative percentage survival and provides the data for Fig. 1. The graph depicts the percentages of women still alive year by year up to the forty-second year after eclampsia. The greatly increased remote mortality rate among multiparous women is evident at a glance.

Hypertension.

Control series. When we began our follow-up study in 1935, we should have selected matched control women for comparison with the patients surviving eclampsia. Perhaps it is fortunate that we did not, for in our ignorance we would have chosen women all of whom had had normotensive pregnancies. We now know that many women who eventually develop essential hypertension manifest the diathesis by elevations in blood

Table VI. Life table for 59 white and 5 black women who had eclampsia as multiparas

Follow-up (yr.)	*No. alive at beginning of year*	*Reason withdrawn*		*Estimated proportion*		*Cumulative survival (%)*
		End of follow-up	*Death*	*Surviving*	*Dying*	
1	64	0	1	0.984	0.016	98.4
2	63	0	1	0.984	0.016	96.8
3	62	0	1	0.984	0.016	95.2
4	61	0	3	0.951	0.049	90.6
5	58	0	1	0.983	0.017	89.0
6	57	0	0	1.000	0.000	89.0
7	57	0	0	1.000	0.000	89.0
8	57	0	0	1.000	0.000	89.0
9	57	0	0	1.000	0.000	89.0
10	57	0	1	0.982	0.018	87.5
11	56	0	1	0.982	0.018	85.8
12	55	0	0	1.000	0.000	85.8
13	55	0	0	1.000	0.000	85.8
14	55	0	0	1.000	0.000	85.8
15	55	0	2	0.964	0.036	82.6
16	53	0	0	1.000	0.000	82.6
17	53	0	1	0.981	0.019	81.0
18	52	0	1	0.981	0.019	79.5
19	51	0	1	0.980	0.020	77.9
20	50	0	0	1.000	0.000	77.9
21	50	0	0	1.000	0.000	77.9
22	50	0	4	0.920	0.080	71.7
23	46	3	1	0.978	0.022	70.2
24	42	1	2	0.952	0.048	66.7
25	39	2	1	0.974	0.026	64.9
26	36	1	0	1.000	0.000	64.9
27	35	1	1	0.971	0.029	63.0
28	33	1	0	1.000	0.000	63.0
29	32	1	2	0.936	0.064	59.0
30	29	1	2	0.930	0.070	54.9
31	26	0	2	0.923	0.077	50.7
32	24	2	0	1.000	0.000	50.7
33	22	0	2	0.909	0.091	46.0
34	20	0	2	0.900	0.100	41.4
35	18	3	2	0.879	0.121	36.4
36	13	1	1	0.920	0.080	33.5
37	11	0	1	0.909	0.091	30.4
38	10	0	0	1.000	0.000	30.4
39	10	2	1	0.889	0.111	27.0
40	7	1	0	1.000	0.000	27.0
41	6	3	0	1.000	0.000	27.0
42	3	3	0	1.000	0.000	27.0

pressure during late gestation. Thus, the selection of women with normotensive pregnancies gives a biased sample in which the eventual prevalence of essential hypertension is seriously underrepresented.

There are several surveys of blood pressure in relation to age in the literature, but there are elements of selection in many of them. Those dealing with people who either had or had applied for life insurance discriminate against hypertensive subjects. The same may be the case, to a lesser extent, in the data of Master, Dublin, and Marks,[28] which are based upon women having or applying for jobs in defense plants and army airfields. Wetherby[44] analyzed the distribution of blood pressures in "practically unselected" outpatients visiting the University Hospital in Minneapolis. He excluded only cases of aortic insufficiency and definite glomerulonephritis; hypertension might be overrepresented in his series. Saller[37] gathered his data in the outpatient clinic in Kiel; he excluded subjects with recognized diseases of the kidneys and circulatory systems, those with anemia, and those with diabetes or other hormonal disorders. Hamilton and co-workers,[18] in London, made a similar analysis of blood pressures in women visiting the outpatient clinics for varicose veins, cutaneous diseases, fractures, orthopedic diseases, and dental treatment. The exclusion of women attending the medical clinic avoided overrepresentation of hypertensive subjects and may have discriminated against them to some extent.

Among epidemiologic studies that seem to be

Table VII. Observed and expected numbers of cases of hypertension at follow-up in women having had eclampsia in the first pregnancy carried to viability

	Systolic pressure		*Diastolic pressure*	
	140+	*160+*	*90+*	*100+*
Number observed	124	63	94	46
Number expected:				
Wetherby[44]	121	81	100	52
Gover[16]	135	90	115	63
Bøe et al.[2]	149	94	104*	42*
Hamilton et al.[18]	119	71	92	46
	144+	164+	94+	104+
Number observed	96	49	56	23
Number expected:				
Saller[37]	94	51	65	29

*Diastolic pressures recorded at phase V; ours were recorded at phase IV.

Table VIII. Observed and expected numbers of cases of hypertension at follow-up in women having had eclampsia as multiparas

	Systolic pressure		*Diastolic pressure*	
	140+	*160+*	*90+*	*100+*
Number observed	50	40	44	27
Number expected:				
Wetherby[44]	40	28	33	18
Gover[16]	45	31	37	21
Bøe et al.[2]	49	35	33*	14*
Hamilton et al.[18]	41	26	31	16
	144+	164+	94+	104+
Number observed	47	37	28	20
Number expected:				
Saller[37]	32	25	22	10

*As in Table VII.

representative of populations are those of Gover,[16] Comstock,[12] Bøe, Humerfelt, and Wedervang,[2] and Kagan and co-workers,[23]. Gover[16] and Comstock[12] studied rural populations, Gover from 19 counties scattered throughout the United States and Comstock from Muscogee County, Georgia. Comstock recorded the third and usually lowest of three readings of the blood pressure and the numbers of cases in the age groups relevant to our study are too small for our use. Bøe, Humerfelt, and Wedervang recorded the blood pressures of 82.4 per cent of the women in Bergen, Norway. In our previous report we[9] used the data of Kagan and co-workers as a control series. In their prospective study of coronary cardiac disease, they recorded casual blood pressures in 71.6 per cent of a

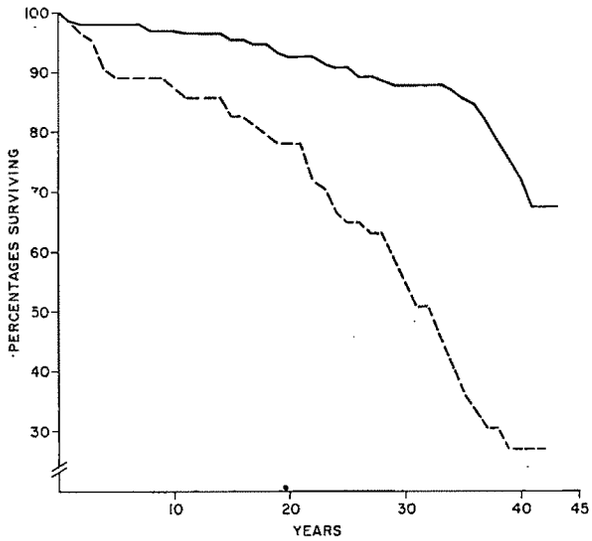


Fig. 1. Survival following eclampsia. The solid line represents women who had eclampsia in the first pregnancy carried to viability; the broken line, those who had eclampsia as multiparas.

random selection of two thirds of the women ages 29 to 62 living in Framingham, Massachusetts. Now, however, many of our primiparous eclamptic women are over age 62, as are nearly half of the women who had eclampsia as multiparas.

Expected vs. actual prevalences of hypertension. The age-specific prevalences of various levels of blood pressure were derived from each of five epidemiologic studies cited. They were then multiplied by the numbers of our patients in each age group, such as 50 to 54, to obtain the expected numbers with given blood pressures. In addition to using blood pressures obtained in 1973-74, we have included the pressures recorded as varying intervals before death in many of the women who have died; pressures taken during terminal sickness were not used. Also, in a few cases not re-examined in 1973, we have used pressures recorded in 1966 if the woman was then aged 50 or more. In all we have usable blood pressures in 197 of the 206 primiparous and 61 of the 64 multiparous eclamptic women.

Table VII compares the numbers of observed primiparous eclamptic women with systolic and diastolic hypertension, by various criteria, with the numbers to be expected from the five epidemiologic studies. The observed prevalence of hypertension is similar to the expected. All of the data are in the table; if, for instance, a systolic pressure of 160 mm. Hg be taken as the upper limit of normal, the observed prevalence of hypertension is slightly less than expected. If 164 mm. Hg be taken as the dividing line,

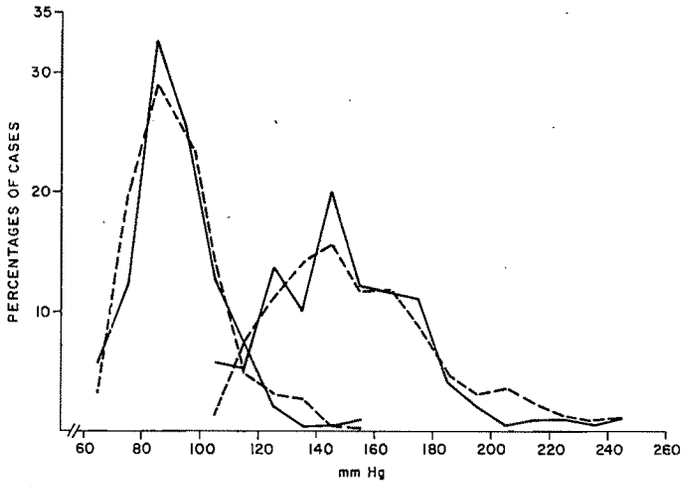


Fig. 2. The distributions of systolic and diastolic blood pressures in women who had eclampsia in the first pregnancy carried to viability (solid lines) as compared with the distributions to be expected from the epidemiologic study of Hamilton and co-workers[18] (broken lines).

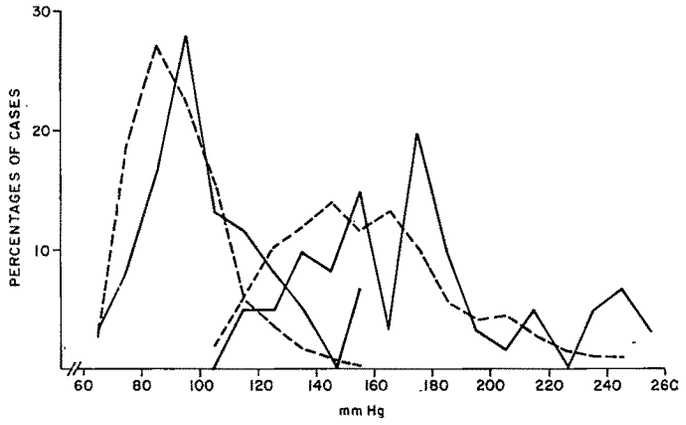


Fig. 3. The distributions of systolic and diastolic blood pressures in women who had eclampsia as multiparas (solid lines) as compared with the distributions to be expected from the epidemiologic study of Hamilton and co-workers[18] (broken lines).

the observed number of cases is 49 as compared with 51 expected from Saller's[37] data. Similarly, the observed numbers of cases with diastolic hypertension, as defined by four levels of pressure, do not differ significantly from the expected numbers. In only two instances are there excesses of observed over expected numbers; with the dividing line set at 90 mm. Hg, the observed number is 94 as compared with 92 to be expected from the data of Hamilton and co-workers[18]; with the dividing line at 100 mm. Hg, the observed number is 46 as compared with 42 to be expected from the data of Bøe, Humerfelt, and Wedervang.[2] (They recorded the lowest observed pressure of several readings and used Phase V as marking the diastolic pressure, whereas we made single readings of casual blood pressures and used phase IV.)

In summary, Table VII indicates that women who had eclampsia as primiparas do not differ from unselected women in the prevalence of hypertension at more than 30 years after eclampsia. It should be mentioned that 96.3 per cent of the blood pressures of our subjects, living or now dead, were recorded when the women were aged 40 or more; 90 per cent at age 45 or more, and 81 per cent at age 50 or more. Few now normotensive are likely to develop essential hypertension.

Multiparous eclamptic women. Table VIII compares the observed numbers of cases with systolic and

Table IX. Relation between chronic hypertension and the nature of pregnancies following eclampsia in primiparas*

Group	*Women still living*	*Women now dead*	*Totals*
No later pregnancies (cases)	36	10	46
Diastolic pressure			
90-99 mm. Hg (%)	22.2	10.0	19.6
100+ mm. Hg (%)	30.6	20.0	28.3
Later pregnancy (total cases)	125	26	151
Diastolic pressure			
90-99 mm. Hg (%)	24.0	34.6	25.8
100+ mm. Hg (%)	21.6	23.1	21.8
All later pregnancies normal (cases)	87	13	100
Diastolic pressure			
90-99 mm. Hg (%)	21.8	46.2	25
100+ mm. Hg (%)	11.5	0	10.0
At least one later hypertensive pregnancy (cases)	38	13	51
Diastolic pressure			
90-99 mm. Hg (%)	29.0	23.1	27.5
100+ mm. Hg (%)	44.7	46.2	45.2

*Nine women omitted because their blood pressures before death are unknown or because the pressures were not measured in recent years. In a few cases not examined in 1973-74, blood pressures recorded in 1966 were used, but only if the women were aged 50 or more at that time.

diastolic hypertension, by various criteria, with the expected numbers. Systolic pressures of 140 mm. Hg or higher are so common in women in the age groups represented by our patients that the comparison is indeterminate (83.6 per cent of our subjects were aged 50 or more when the blood pressures were recorded; 37.8 per cent were aged 60 to 69; and 13 per cent were aged 70 to 82).

When we look at the diastolic pressures, however, we see a clear excess of hypertensive cases over the expected numbers derived from each of the five control series and at any of the four levels of pressure taken as defining hypertension. The excess of hypertensive cases does not mean that eclampsia caused the hypertension, for the multiparous eclamptic group is heavily weighted with women who had antecedent hypertension that predisposed them to eclampsia in the first place.

As may be seen in Table VII and VIII, the data of Hamilton and co-workers[18] give the lowest expected numbers of hypertensive cases, with a single exception: at a dividing line of 100 mm. Hg in the diastolic pressure, the data of Bøe, Humerfelt, and Wedervang[2] give two or four fewer cases. We have, therefore, used the data of Hamilton as the most rigid of the several control series in comparing the distributions of systolic and diastolic pressures of posteclamptic women with those of unselected urban women matched for age. Fig. 2 shows that the distribution of pressures of women who had eclampsia as primiparas, at an average of more than 30 years later, is not different from that in the control population. In contrast, Fig. 3 indicates a considerable excess of hypertensive cases among the women who had eclampsia as multiparas. As with remote death rates and causes of death, the multiparous are different from the primiparous eclamptic women.

Prognostic significance of recurrent hypertensive pregnancies. In an earlier report based upon the findings in 1959, we[8] observed that primiparous eclamptic women who had later pregnancies were separable into two groups. Many of the one third with at least one later hypertensive pregnancy eventually developed chronic hypertension, whereas few of the two thirds whose later pregnancies were all normotensive did so. Moreover, the women with normotensive pregnancies were remarkable in that the prevalence of hypertension did not seem to increase with age, which must require some special explanation. Our interpretation was that the later pregnancies were a screening test for the hypertensive diathesis: those who ultimately would develop chronic hypertension manifested it by transient gestational hypertension, whereas the ever normotensive women had normotensive pregnancies.

Now that 94.4 per cent of the primiparous eclamptic women still living are aged 45 or more, nearly all destined for essential hypertension might be expected to have developed it. We have, therefore, re-examined the relation between chronic hypertension and the nature of the pregnancies subsequent to eclampsia in

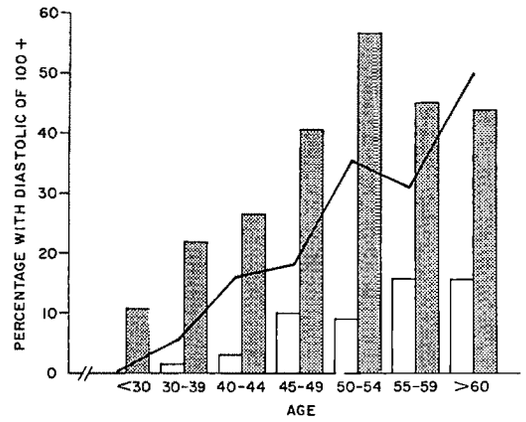


Fig. 4. Hypertension at follow-up in relation to age and the nature of pregnancies subsequent to eclampsia in primiparas. Solid line represents 46 women with no later viable pregnancy; open bars represent 100 women whose later pregnancies were normotensive; stippled bars represent 51 women who had at least one later hypertensive pregnancy. Each woman is represented by an average of 4.4 blood pressures measured at intervals of 7 years.

the first gestation carried to viability. Table IX sets out one approach. In the 46 women having had no later pregnancy carried to viability, the prevalence of diastolic pressures of 100 mm. Hg or higher is 28.3 per cent as compared with 21.8 per cent in the 151 women who had from 1 to 11 later viable pregnancies. (The difference is not significant; $\chi^2 = 0.81$, p is about 0.4). Inasmuch as a full third of the women having later pregnancies developed gestational hypertension at least once, it appears that repeated hypertensive gestations (eclampsia in the first and hypertension in a later one) do not cause chronic hypertension.

Diastolic pressures of 100 mm. Hg or higher were found in only 10 per cent of the 100 women whose later pregnancies all were normotensive, as compared with 45 per cent in the 51 women who had a later hypertensive pregnancy. The difference is highly significant: ($\chi^2 = 24.4$, $p < 0.001$). Recurrent gestational hypertension, therefore, is often a sign of future essential hypertension although 55 per cent of the women with such pregnancies are still normal at follow-up.

We have not been able to discern any pattern in the development of hypertension in pregnancies following eclampsia. The recurrent hypertension may occur in only one of several gestations, or in two, or in more. There seems to be no predilection for any one later pregnancy, such as the first after eclampsia. Over all, one third of the primiparous eclamptic women having later pregnancies carried to viability have developed hypertension in 20 per cent of their gestations. Gestational hypertension was the only finding in about 40 per cent of the cases; proteinuria occurred in less than half and, if present, often was minimal, although eight women had what appeared to be severe preeclampsia and four had a second eclampsia.

Fig. 4 is another approach to the question as to the relation between chronic hypertension and recurrent gestational hypertension. The graph is based upon 444 readings of the blood pressure in the 100 women whose later pregnancies were all normotensive and upon 225 readings in the 51 women who had at least one later hypertensive pregnancy; the readings in each woman were made at intervals of 7 years, going back to 1935 for those who had had eclampsia that early. In the category shown in the graph as less than 30 years of age, only the last reading was recorded; for instance, a woman having had eclampsia at age 16 was reexamined several times before she attained age 30, but only the last recording was used in the analysis.

As may be seen in Fig. 4, the prevalence of diastolic pressures of 100 mm. Hg or higher, at any age, was markedly greater among the women who had had recurrent gestational hypertension than among those who escaped it. The decrease of about 12 per cent after age 54 is attributable to early deaths. As shown in Table III, 13 of the remote deaths were in the cardiovascular field; nine occurred in women who had had recurrent gestational hypertension, two in women having had no later pregnancies, and two in women all of whose later pregnancies had been normotensive.

Our earlier conclusion that the prevalence of chronic hypertension does not increase with age in primiparous eclamptic women whose later pregnancies were all normotensive was based upon blood pressures and ages of the women as we found them in 1959; each of the survivors is now 14 years older. In a sense, our earlier study was cross-sectional as distinguished from the serial study represented in Fig. 4, in which the trend of each woman's blood pressure over a period of from 22 to 42 years is inherent. Our original conclusion was erroneous; as Fig. 4 shows, the prevalence of hypertension does rise with age in the group with normotensive pregnancies, but not nearly so markedly as in the women who had gestational hypertension subsequent to eclampsia. Moreover, the prevalence does not increase so rapidly with age as in the unselected women in the epidemiologic studies cited, nor does it attain even half of the expected level.

We also had suggested that recurrent gestational hypertension might precipitate prematurely a permanent hypertension that was in the making. Fig. 4 could be interpreted in that way. The solid line shows the prevalence of chronic hypertension in the 46 women

Table X. Diabetes in women having had eclampsia

Age at death or follow-up	*Prevalence per 1,000*	*Primiparous eclamptic women*		*Multiparous eclamptic women*	
		Cases	*Expected diabetes*	*Cases*	*Expected diabetes*
15 to 24	1.7	2	0.003	0	0
25 to 44	7.9	18	0.140	8	0.063
45 to 64	31.0	147	4.464	36	1.116
65+	74.9	26	1.945	19	1.423
Totals		193*	6.6552	63*	2.602
Observed			17		10
Ratio, actual/expected			2.5		3.8

*Thirteen primiparous and one multiparous eclamptic women not re-examined in 1973-74.

Table XI. The detection of diabetes after eclampsia

	Primiparous eclamptic women		*Multiparous eclamptic women*	
	Age at detection	*Years after eclampsia*	*Age at detection*	*Years after eclampsia*
Range	36-67	14-35	42-65	3-32
Mean	51	25	55	17
Median	49	24	55	19

who had no viable pregnancies following eclampsia; it reached a maximal level of 50 per cent in the women aged 60 or more (22 cases in that age group). The maximal prevalence of hypertension in the women with recurrent gestational hypertension was about the same at 56 per cent, but was found at age 50 to 54, or 10 years earlier (23 cases in that age group). Moreover, the prevalence of hypertension was higher in each age group of those with recurrently hypertensive pregnancies than among women having had no later pregnancies. Diastolic pressures of 100 mm. Hg or higher were found before age 30 in 11.1 per cent of the women who had had recurrent gestational hypertension. In contrast, the findings in the women having had no later pregnancies were as follows: before age 30, none with a diastolic pressure as high as 100 mm. Hg (or even as high as 90); ages 30 to 34, 3.8 per cent; ages 35 to 39, 6.5 per cent; and ages 40 to 44, 16 per cent.

Fig. 4 also shows the screening effect of later pregnancies, for the prevalence of chronic hypertension in women having had no later pregnancies is intermediate between that in women whose gestations were all normotensive and those who had had a later hypertensive pregnancy. The nature of the later pregnancies separates fairly well the women likely and unlikely to develop chronic hypertension.

The late development of diabetes. Diabetes was not recognized in any patient during her eclamptic pregnancy. In our follow-up study in 1966, we[9] found that 20 women had developed diabetes, which was about seven times the expected number. The expected number was calculated from the age-specific prevalences set out in the ninth edition of *The Treatment of Diabetes Mellitus*[22] and they appear to have been underestimates. In the eleventh edition, *Joslin's Diabetes Mellitus,*[26] the age-specific prevalences shown are nearly three times higher, but that still would leave an excess of cases among the women who had had eclampsia.

We wrote to each of our patients in 1967, urging them to have annual tests for diabetes; seven new cases have been detected since then and the count now stands at 17 cases among the primiparous and 10 cases among the multiparous eclamptic women. One woman, who was normal in several examinations before 1952, refused in 1952 and since. Five weeks after her refusal in 1967 she was admitted to another hospital in diabetic coma and died.

As Table X shows, the prevalence of diabetes is about 2½ and 4 times the expected rate among primiparous and multiparous eclamptic women, respectively. Table XI indicates that diabetes was detected at from 3 to 35 years after eclampsia, at an average age in the early 50's (range of ages, 36 to 67) .

Comment

Table VII and Fig. 2 show that primiparous eclamptic women do not differ from unselected control women in the prevalence of hypertension many years after eclampsia. Nearly all have attained ages in which the future development of essential hypertension is unlikely, whence one must conclude that eclampsia is not a sign of the hypertensive diathesis.

Moreover, the normal (expected) prevalence of hypertension indicates that eclampsia and later hypertensive pregnancies have not caused hypertension in women who otherwise would never have developed it. A third (51) of the 151 having later pregnancies had gestational hypertension at least once following eclampsia, yet the prevalence of chronic hypertension

Table XII. Comparison of primiparous and multiparous eclamptic women*

Author	Year	Length of follow-up	Primiparas		Multiparas	
			Cases	Hypertension (%)	Cases	Hypertension (%)
Peckham[34]	1929	13 mo.	49	12.2	28	39.3
Breakey[3]	1932	Up to 30 yr.	59	11.9	35	45.8
Bryans[4]	1966	Mean 24.1 yr.	58	44.6	60	61.7
Chesley et al.[9]	1968	15-35 yr.	195	46.1	64	73.6

*Differing standards for hypertension, but the same one used in each comparison.

among the 151 is not increased over that in the 46 women who had no later viable pregnancy. Actually, the prevalence is lower among those with later gestations, although not significantly so (diastolic pressures of 100 mm. Hg or higher in 21.8 and 28.3 per cent, respectively; Table IX). If repeated gestational hypertension causes chronic hypertension, the women with later pregnancies should have an increased prevalence because one third is too large a fraction to be lost in the averages.

Four observations support our earlier conclusion that pregnancies following eclampsia constitute a screening test for the hypertensive diathesis. (1) Later hypertensive pregnancies do not cause chronic hypertension, as discussed in the preceding paragraph. (2) The prevalence of hypertension at follow-up is far smaller than expected among women all of whose later pregnancies have been normotensive. (3) The prevalence of hypertension at follow-up is far greater than expected among women who have had at least one later hypertensive pregnancy. (4) The prevalence of chronic hypertension among women having had no later viable pregnancy is intermediate between those in the two groups with later pregnancies; that is, women with no later pregnancy have not been separated by the test into groups likely and unlikely to develop essential hypertension.

Recurrent gestational hypertension is often a sign of future essential hypertension and, for that particular group of hypertensive disorders in pregnancy, Fishberg[13] and Herrick and Tillman[19] appear to have been correct in attributing that significance to hypertension in pregnancy. As mentioned before, the recurrent gestational hypertension is usually mild and is usually accompanied by little or no proteinuria. In most clinics the diagnosis is likely to be "mild pre-eclampsia," but it is not pre-eclampsia. Moreover, if pregnancies following eclampsia evoke gestational hypertension in women with the hypertensive diathesis, why should not the first pregnancy do so in women who escape eclampsia? We believe that what is called mild pre-eclampsia in some primigravidas is, in fact, not pre-eclampsia but rather is latent essential hypertension brought to light by pregnancy. For that group, again, Fishberg[13] and Herrick and Tillman[19] were correct.

The foregoing considerations seem to explain why nearly all investigators have found a higher prevalence of chronic hypertension following "mild pre-eclampsia" than after eclampsia. They also bear upon the confusion introduced by the inclusion of multiparas in any analysis of the relation between pre-eclampsia and essential hypertension. The acute onset of mild hypertension with edema and perhaps a little proteinuria in a primigravida is likely to be pre-eclampsia, although not necessarily so. The same syndrome in a multipara is far less likely to be pre-eclampsia, especially if she has had an earlier hypertensive pregnancy.

The differing prognoses for primiparous and multiparous eclamptic women have been established in the tables and figures and the difference pertains to remote average annual death rates, causes of death, and the prevalences of hypertension and diabetes. Some authors recognized the difference years ago, but most have continued to combine all cases of eclampsia in their reports. Table XII samples the literature; Peckham's[33] diagnoses were "chronic nephritis" without specification of the criteria. Breakey's[3] diagnoses, also, were "chronic nephritis" but she tabulated numbers of cases with systolic pressures of 160 mm. Hg or higher. She reported that remote deaths from "nephritis" occurred in five multiparous eclamptic women, whereas there were none in the primiparous group. In our present study the remote average annual death rate in multiparous eclamptic women was four times greater than in the primiparous group. Moreover, the percentages of deaths attributable to cardiovascular diseases were greatly different, being 29 and 82 per cent in white primiparous and multiparous eclamptic women, respectively

Bryans[4] and Tillman[43] have reported studies that must be considered in any discussion of the relation between pre-eclampsia–eclampsia and chronic hyper-

tension. Bryans[4] has followed more eclamptic women (335) for longer (up to 44 years) than has anyone else. He has concluded that eclampsia does not cause essential hypertension because its prevalence is not increased in posteclamptic women over that found in epidemiologic studies of comparable populations. He has gone a step further in recording blood pressures of relatives of the eclamptic women and has found that a proved familial history of hypertension is more frequent among previously eclamptic women who develop hypertension than among those who remain normotensive.

Tillman[43] accumulated a series of 377 women whose blood pressures were recorded before their first pregnancies. Of the first pregnancies, 240 were normotensive and 137 were complicated by gestational hypertension. Follow-up studies indicated that the blood pressures had not been affected by normal or hypertensive pregnancies, even among those who had been hypertensive before conception.

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Production of experimental toxemia in the pregnant rabbit

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Experimental toxemia of pregnancy was induced in 51 of 122 rabbits by constriction of the aorta below the renal arteries. The approach was extraperitoneal and dependent on the accuracy in calibrating this stricture between 0.6 and 1.0 mm. Experimental toxemia in the rabbit was characterized by hypertension, proteinuria, weight gain, and reduced weight of the fetus. Blood pressure and blood flow studies distal to the aortic constriction demonstrated a marked diminution of blood supply below the constriction. The light microscopic changes in the kidneys and in the liver were similar to those of human toxemia. The electron microscopic changes consisted of endothelial swelling and subendothelial deposits. In a separate experiment, 22 pregnant rabbits near term had an aortic constriction varying between 0.6 and 1.0 mm. This constriction lasted 4 to 12 days. Glomerular deposits of fibrinogen were demonstrated by immunofluorescence in 20 of 22 animals. The intensity of the immunofluoresence was related to the severity and duration of the aortic stricture.

THE CONCEPT that ischemia of the pregnant uterus is responsible for toxemia was first proposed by Young[1] in 1927. Many authors were subsequently influenced by the concept of Goldblatt and associates[2] that, if chronic renal ischemia leads to hypertension, then chronic placental ischemia may possibly lead to toxemia. Beker[3] was the first to report radiologic evidence of diminished blood flow to the toxemic placenta. In 1940 Ogden, Hildebrand, and Page[4] produced blood pressure elevation in pregnant dogs by compressing the aorta below the renal arteries. Van Bouwdijk, Bastiaanse[5] and Douglas[6] satisfactorily repeated the same experiment. Gyöngyössy and Kelentey[7] raised the blood pressure of pregnant dogs and cats by producing intrauterine pressure with balloons. Kumar[8] in 1962 reported transitory proteinuria in addition to hypertension following constriction of the uterine and ovarian arteries of pregnant dogs. Berger and Cavanagh[9] in 1963 also produced hypertension in pregnant rabbits by interfering with the uterine and placental circulation. In 1967 Douglas and Langford[10] produced hypertension in the rat and proteinuria in the rabbit by wrapping the pregnant uteri of these animals. In 1969 Hodari[11] produced chronic ischemia in the pregnant uterus in dogs by preventing the physiologic dilatation of the uterine artery during pregnancy; he achieved this by pre-pregnancy placement of a snug-fitting hard Teflon band around the uterine arteries, producing hypertension, proteinuria, and hypernatremia. Cavanagh and associates[12] have recently reported similar results in the baboon.

Doubts have been cast on the above experiments, however, since some of the results were not confirmed in other laboratories. Still, there is a large body of clinical evidence to suggest that the placenta is ischemic during toxemia and may produce a hyperten-

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Table I. Degrees of aortic stricture

Group No.	*Degree of aortic stricture*	*No. of rabbits*	*Condition of pregnancy*	*Pathologic manifestations*
I	Between 0.1 and 0.3 mm.	16	6 Not pregnant 10 Pregnant	Paralysis of hind legs Hypotension, occasional Proteinuria Renal tubular necrosis
II	Between 0.3 and 0.6 mm.	11	11 Pregnant	Placental separation Mild and transient proteinuria or hypertension in some rabbits
III	Between 0.6 and 1.0 mm.	63	12 Not pregnant 51 Pregnant	None Proteinuria, hypertension Renal and/or hepatic lesions
IV	Between 1.0 and 1.3 mm.	7	7 Pregnant	Mild and transient proteinuria or hypertension in some rabbits
V	Between 1.3 and 3.0 mm. (sham operation)	17	17 Pregnant	None
VI	No operation	8	3 Not pregnant 5 Pregnant	None

sive factor, the nature of which is still under investigation.[13, 14]

The etiology of toxemia remains obscure. Studies to elucidate pathogenetic mechanisms provide evidence to suggest abnormalities of the clotting mechanism[15–17] and experimentally in rabbits infusion of thromboplastin produced glomerular lesions similar to those seen in toxemia.[18] Although initiating factors are unknown, one central hypothesis holds that ischemia of the uteroplacental unit stimulates release of biologically active substances into the maternal circulation promoting glomeruler lesions.[19] Morphologic alterations seen in glomeruli of human subjects with toxemia include endothelial cell swelling by light microscopy[20] and dense deposits between endothelial cells and glomerular basement membrane by electron microscopy.[21–25] These deposits have been identified as fibrin by immunofluorescence.[26, 27]

This report describes experimentally induced lesions in pregnant rabbits.

Material and methods

New Zealand rabbits weighing 7 to 9 pounds were used for these experiments. Of 122 rabbits, 101 were pregnant and 21 were not (Table I). Pregnancy in the rabbit lasts between 28 and 32 days, and these animals were between 14 and 24 days of gestation at the time of operation.

After induction of anesthesia, a left paralumbar incision was made and the abdominal aorta was approached extraperitoneally. The vessel was carefully exposed down to the bifurcation into the common iliac arteries. A No. 3 silk suture was then placed loosely around the aorta, 1 cm. below the renal arteries. While the silk suture was progressively tightened, the finger of the operator was placed gently on the aorta about 1 cm. below the point of constriction. When the finger could feel a bruit through the stricture, a second and third silk knot were placed, to secure the constriction at that specific degree.

The animals were checked daily for weight and blood pressure for 2 days before surgery, then every other day thereafter. Urine was checked for protein whenever possible with the sulfosalicylic acid method.

The technique of the Grant-Rothschild capsule on the rabbit's ear was used to measure blood pressure. This apparatus permits only the measurement of the systolic pressure. The ear blood pressure was taken under rigidly controlled environmental conditions: the measurement was taken by the same person each day in the early morning, when the animal room was free of any noise. After the animal had been quieted down (respiration not rapid) and warmed, the ear pressure was measured consecutively five times; if there was, among these five pressures, a difference of less than 10 mm. Hg, the average was calculated and registered as the pressure for that day; if the difference was higher than 10 mm. of mercury, it usually meant that the animal was still excited, and the measurements were repeated 15 minutes later.

Two to 14 days after the operation or immediately after delivery (if spontaneous), the animals were put to death. The abdominal aorta was exposed at the point of constriction. The intact silk knot was carefully removed, cleaned, placed between two slides, and examined by low-power microscopy with a calibrated

Table II. Blood pressure variations and standard deviations

Group No.	*Degree of aortic stricture (mm.)*	*No. of rabbits*	*Condition of pregnancy*	*Average B.P. before surgery*	*Average B.P. after surgery*	*Change in B.P.*
I	0.1-0.3	16	6 Not pregnant	73.5 ± 3.0	48.7 ± 12.6	−24.8
			10 Pregnant	69.7 ± 6.3	52.6 ± 9.7	−17.1
II	0.3-0.6	11	11 Pregnant	68.8 ± 4.7	72.0 ± 7.7	+ 3.2
III	0.6-1.0	63	12 Not pregnant	80.1 ± 4.2	79.3 ± 6.2	− 0.8
			51 Pregnant	71.4 ± 5.6	91.9 ± 8.9	+20.5
IV	1.0-1.3	7	7 Pregnant	71.2 ± 3.7	72.8 ± 6.9	+ 1.6
V	1.3-3.0 (sham operation)	17	17 Pregnant	70.1 ± 5.4	73.8 ± 7.2	+ 3.7
VI	No operation	8	3 Not pregnant	82.1 ± 3.6		
			5 Pregnant	67.6 ± 6.1		

micrometer. The inner diameter of the knot could be easily measured to 0.1 mm. and the value thus obtained represented the constriction of the aorta for that specific animal. With this method, it was only after the rabbit had been killed that the degree of constriction of the abdominal aorta could be measured accurately. Different strictures varying from 0.1 to 3 mm. were recorded, and this allowed the classification of the 122 rabbits into six different groups as shown in Table I.

Nonpregnant animals from Group III and all the animals from Groups IV, V, and VI were considered controls. When each animal was killed, a specimen of blood from the heart and urine from the bladder was taken. Blocks from the following organs were fixed in 10 per cent formalin for histologic studies: liver, kidneys, adrenals, spleen, uterus, placenta, heart, lungs, and brain. Sections were stained with hematoxylin and eosin (H & E), alcian blue (AB), and periodic acid–Schiff (PAS). For electron microscopy, small samples of renal cortex were fixed in 2.5 per cent glutaldehyde, post-fixed in osmium tetraoxide (OsO_4). Ultrathin sections were stained with uracyl acetate and lead citrate and observed in a Hitachi HS8 electron microscope at 50 KV.

Results

Except for the 51 pregnant rabbits from Group III, the pathologic manifestations in the other groups were minimal.

1. The animals from Group I which had a severe aortic stricture, varying between 0.1 and 0.3 mm., developed an immediate paralysis of the hind legs, hypotension, and occasional proteinuria. They died spontaneously or were killed within 2 days. The only significant pathologic change was renal tubular necrosis; the renal glomeruli and the liver were normal.

2. The animals from Group II, which had an aortic stricture varying between 0.3 and 0.6 mm., had a rapid fetal death with separation of the placenta, transient and mild hypertension, or proteinuria; histologic study of the liver and kidneys did not show any abnormality.

3. There were no clinical or morphological abnormalities in the 12 nonpregnant rabbits from Group III, or any other animals from Groups IV, V, and VI. Mild and transient proteinuria or hypertension were occasionally noted; all these animals were, therefore, considered as controls.

Consistent and significant pathologic manifestations developed only with the combination of pregnancy and an aortic stricture varying between 0.6 and 1.0 mm. These clinical and pathologic manifestations exhibited by the 51 pregnant rabbits from Group III are described below in detail.

Clinical findings.

Blood pressure. The average blood pressure and standard deviations for all animals are recorded in Table II. Normal systolic pressure in the nonpregnant rabbit varied between 70 and 90 mm. Hg. In pregnant rabbits the average systolic pressure before the operation varied from 50 to 85 mm. Hg. After the operation, each of the 51 pregnant animals from Group III presented a rise in blood pressure; in some animals the rise was as high as 35 and in others, it was as low as 10 mm. Hg. The average blood pressure of these animals was 71.4 mm. Hg before surgery; then it rose suddenly to 89.9 mm. Hg on the first postoperative day, a highly significant increase ($p < 2 \times 10^{-9}$). These rabbits were killed 2 to 14 days after the operation. In those which lived beyond 2 days, the blood pressure kept rising slowly, up to an average of 96.2 mm. Hg on the fourteenth postoperative day.

Proteinuria. This test was difficult to perform on a daily basis because of difficulty in obtaining a daily urine specimen; however, enough tests were per-

Table III. Blood pressure and blood flow measurements before and after aortic constriction

Rabbit No.	*Degree of aortic constriction (mm.)*	*Blood pressure in lower abdominal aorta*		*Mean blood flow in hypogastric artery*	
		Before constriction	*After constriction*	*Before constriction*	*After constriction*
1	0	82/72		51	
2	2.0	78/68	74/66	12	11
3	1.1	74/62	65/55	35	32
4	1.1	80/73	82/72	Not recorded	Not recorded
5	0.9	74/65	46/39	39	26
6	0.9	71/64	49/41	20	14
7	0.8	82/68	49/42	58	40
8	0.8	71/61	50/43	Not recorded	Not recorded
9	0.8	77/63	39/32	14	7
10	0.6	Not recorded	Not recorded	12	5
11	0.6	79/72	30/26	17	3
12	0.4	75/64	30/25	Not recorded	Not recorded
13	0.2	73/65	22/18	36	13
14	0.1	75/62	15/11	14	6

formed so that one could discern a general pattern of this abnormality. Among the 51 pregnant rabbits from Group III, after the operation proteinuria was persistently present in 15, transient in 16, and absent or in only trace amounts in the 17 others. Among the 44 animals from the control groups, only a transient trace of proteinuria was detected in four, the urine was completely normal in 35, and was not checked in the remaining five.

Prematurity and fetal deaths. While all the pregnant rabbits from the control groups had a gestation of normal duration, this did not occur in the 51 pregnant rabbits from Group III. Only 29 of them went to term (28 to 32 days), at which time they delivered spontaneously or were killed: 14 had a living litter, or at least showed no signs of maceration; seven had a litter partly alive and partly macerated; eight had the whole litter macerated. The other 22 rabbits delivered prematurely between 17 and 27 days of gestation: in 13 the whole litter either was alive or showed no signs of maceration; three had a litter partly alive and partly macerated; six had the whole litter macerated.

Weight of the living fetuses. The average weight of a newborn rabbit varies from 60 to 70 grams (average, 64 grams). This was determined in the five pregnant rabbits of the last group of this study, as they were killed between 28 and 32 days of gestation without any operation. Among the 51 pregnant rabbits from Group III, seven cases were selected because they had a living litter, and they matched, day by day, the length of gestation of the above control group. Thus, living fetuses of the same length of gestation could be compared between normal pregnancy and pregnancy associated with aortic constriction: the average weight of this experimental group varied between 41 and 62 grams (average, 52 grams). Therefore, expemimental toxemia reduced the mean weight of the fetus at term from 64 to 52 grams.

Weight gain. All the 122 animals studied here were weighed daily. There was no appreciable difference in weight gain among the different groups of pregnant rabbits. However, fluid and electrolyte retention was elicited indirectly: 200 ml. of a normal saline solution were injected daily subcutaneously into six animals from the different control groups and three pregnant rabbits from Group III (aortic stricture between 0.6 and 1.0 mm.). Control animals showed limited or no weight gain, but the three pregnant rabbits from Group III showed sudden weight gains varying from 50 to 250 grams a day.

Blood flow and blood pressure measurements below the aortic constriction. The reduction in blood supply produced below the aortic constriction was measured in a separate experiment on 14 pregnant rabbits near term, all of approximately the same weight. In these animals, the abdominal aorta was approached intraperitoneally and dissected all the way from the renal arteries down to the iliac bifurcation.

Blood pressure in the abdominal aorta was measured by introducing an angiocatheter, 0.7 mm. in diameter, into the aorta just above the iliac bifurcation, and connecting it to a pressure transducer-recorder system. Also, blood flow measurements in the hypogastric artery were taken by placing a 0.75 mm. flow transducer around this vessel, and connecting it to a recorder (blood flow measurements directly around the uterine artery were not satisfactory because of the small diameter of this vessel).

Following placement of the suture, repeat pressures below the constriction were obtained (Table III). After

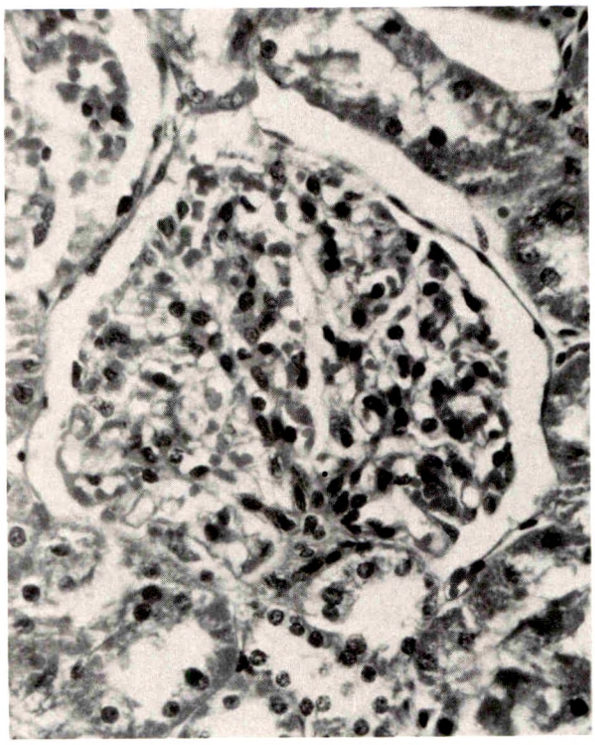


Fig. 1. Glomerulus showing segmental hypercellularity on the right, ischemia and edema of the capillary loops on the left, and mesangial thickening at the base.

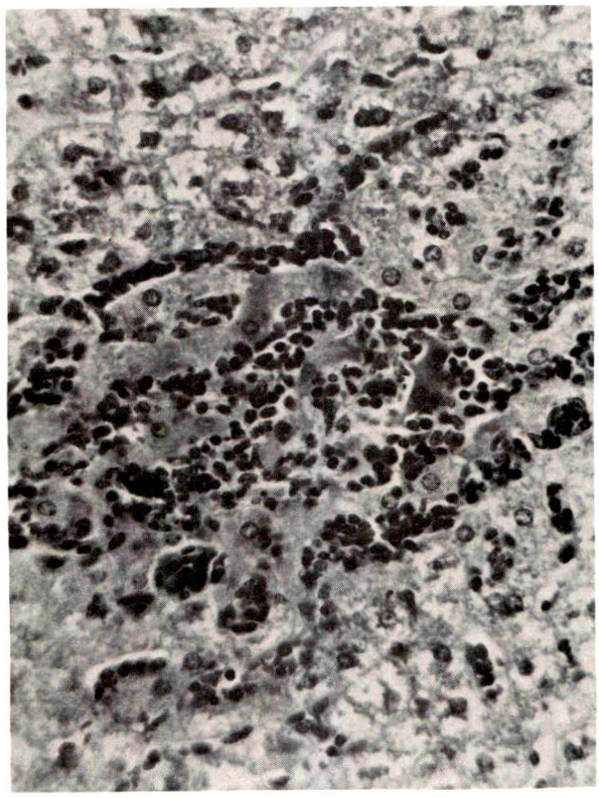


Fig. 2. High-power view of a focal hepatic necrosis showing necrotic hepatocytes and infiltration by polymorphonuclear leukocytes.

the completion of these measurements, the animal was killed; the silk knot that was placed around the abdominal aorta was carefully removed, and its inner diameter measured according to the method described previously.

The blood flow in the hypogastric artery before constriction presented a range of variations between 11 and 58 ml. per minute, depending on the animal; after the constriction the drop in blood flow and in pulse flow (difference between the systolic and the diastolic blood flow) was proportionately similar to the drop in blood pressure and pulse pressure.

Light microscopic findings. While proteinuria and/or hypertension was present in 51 pregnant animals of Group III, only 40 of them had light-microscopic changes in the kidneys or in the liver. The liver was involved in 15 instances and the kidneys in 37 instances.

Kidneys. Apart from the over-all pallor, no gross lesions were visible in the kidneys on either the external or cut surfaces. Microscopic evidence of glomerular lesions was detected in 37 of the 51 cases with clinical evidence of toxemia. The glomerular lesions were mild, infrequent, and not fully developed in animals in which aortic constriction was maintained for only 2 to 4 days prior to death; lesions of greater magnitude were found in a larger proportion of glomeruli in animals in which aortic constriction was maintained for 8 to 15 days before death. Affected glomeruli showed varying degrees of ischemia and edema of the capillary tuft; in some glomeruli the alteration was segmental, involving only a portion of the tuft. In other glomeruli, hypercellularity and edema of endothelial and/or mesangial cells was evident; not uncommonly this change was accompanied by mesangial thickening (Fig. 1). The most significant lesion, however, occurred in or near the basement membrane of glomerular capillary loops, as seen by light microscopy, vi., segmental thickening of the basement membrane, thickened segments alternating with others of normal thinness (Fig. 1); such lesions were readily detectable in sections stained conventionally with H & E and the observation was confirmed by examining parallel sections from the same block stained with PAS and AB.

Liver. Hepatic lesions were less frequent. Apart from an occasional subcapsular petechial hemorrhage, no gross lesions were visible. In 15 of 51 animals with



Fig. 3. Case 8. Representative glomerular capillary with swollen endothelial cell *(EN)* and a relative narrow capillary lumen *(CL)*. There is focal fusion of the foot processes. In the mesangial area *(M)* the cytoplasm of the mesangial cells is "shrunken" and in one area is seen to extend peripherally beneath the endothelium (arrows). (×6,000.)

clinical evidence of toxemia focal necrosis distributed at random and without reference to lobular architecture could be detected (Fig. 2). Foci of necrotic hepatocytes showed coagulated eosinophilic cytoplasm and infiltration by polymorphonuclear leukocytes, but these foci were not hemorrhagic nor were they associated with intrasinusoidal fibrin thrombi. An occasional section demonstrated a small focus of subcapsular hemorrhage with no leukocytic response.

Other organs. The changes in the other organs consisted essentially of interstitital hemorrhage, congestion, and edema; no vascular thrombosis was observed. The placental changes in experimental toxemia will be reported separately.

Electron microscopic findings. The kidneys of 22 rabbits with experimental toxemia and one control rabbit were examined by electron microscopy. The control rabbit and three rabbits with experimental toxemia did not present any abnormality, but 19 animals presented different kinds of abnormalities.

Endothelial cell swelling was observed in 11 rabbits and was either moderate or severe in nine (Figs. 3 and 4). This feature was usually associated with an increased number of cytoplasmic organelles. Mesangial cell swelling was much less common, being present in only two cases. The most striking and common change in the *mesangial cells* was their dark and shrunken cytoplasm, present in eight of the cases and often associated with increased prominence of the intracytoplasmic fibrils and their attachment plates along



Fig. 4. Case 6. Glomerular capillary with swollen endothelial cell completely obliterating the capillary lumen. The foot processes are discrete. (×6,000.)

the plasma membrane. In several instances thin and elongated projections of mesangial cell cytoplasm were seen to extend beneath the endothelium for a short distance from the mesangial areas (Fig. 3). Occasionally, the plasma membrane of these degenerate mesangial cells was indistinct and poorly defined fibrils were seen to extend into or to be free within the mesangial matrix. In a few animals fibers of the approximate size and configuration of collagen fibers were present in the mesangial matrix. The *visceral epithelial cells* were slightly swollen in a few cases. Fusion of foot processes, usually focal and rather mild, was present in the glomeruli of 13 animals (Figs. 3 and 4). No definite abnormalities of the *capillary basement membrane* were observed. Occasionally segmental thickening of the lamina densa and widening of the laminae rarae were noted but both of these changes could generally be attributed to tangential sectioning. *Electron-dense deposits* were definitely present in four animals and questionably in one other case (Fig. 5). They were located in subendothelial or submesangial areas and had a granular texture. No fibrillar structures were recognized in these areas. In the capillary lumen, no coagulation was seen; however, in two animals, a number of platelets were noted in the glomerular capillaries.

Immunofluorescence studies. After the previous experiments had been completed an additional group of animals was studied in order to obtain information as to the presence or absence of fibrinogen-fibrin in the glomeruli.

Material. Of the 32 rabbits in this group, 27 were pregnant and five were not. Experimental toxemia was produced in 22 of the pregnant rabbits following the method previously described: 4 to 12 days before term, the abdominal aorta was constricted to a diameter varying between 0.6 and 1.0 mm. The aortic stricture was considered to be prolonged if it had been placed 7 days or more prior to death. All these animals were killed toward the end of their pregnancy. The only

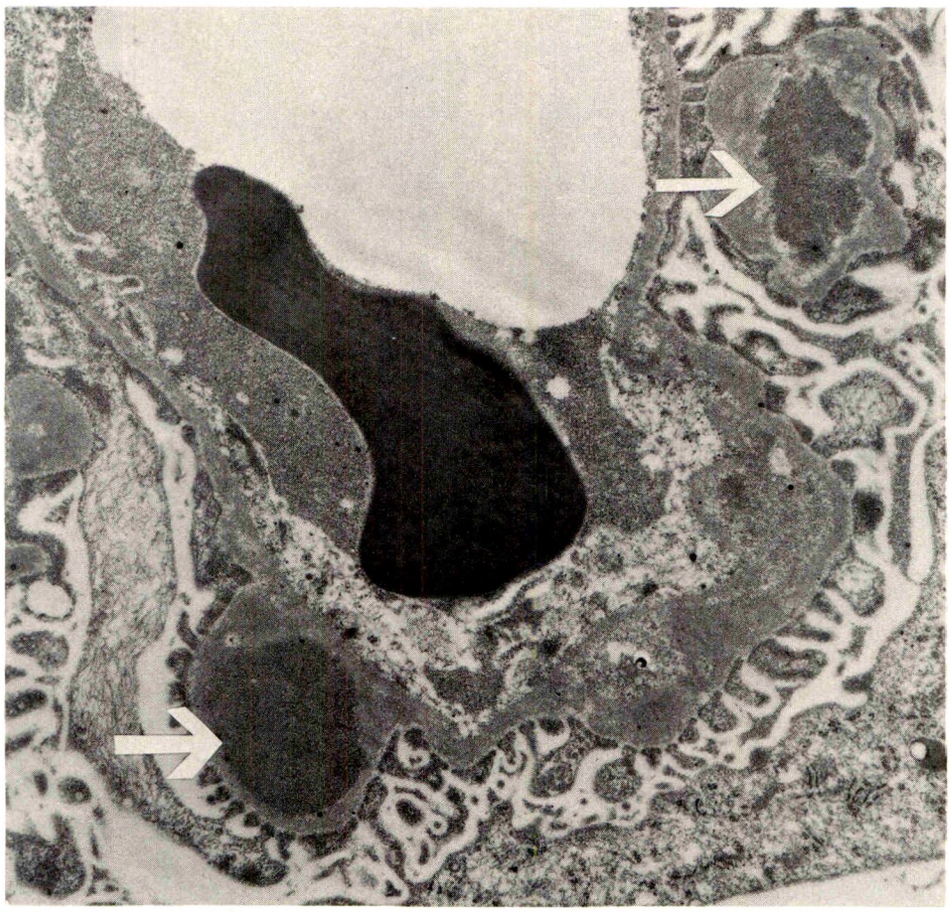


Fig. 5. Case 3. Electron-dense deposits in subendothelial areas (arrows) along the capillary basement membrane (arrows). The endothelial cytoplasm is slightly swollen. There is focal fusion in foot processes. (×7,900.)

exception was rabbit No. 14, which was killed 9 days after spontaneous delivery.

Ten animals served as a control for this immunofluoresence study. (1) Four were pregnant and had a sham operation 4 to 9 days before being killed. In these animals the abdominal aorta was mildly constricted. (2) Two were pregnant and were killed at 30 days of pregnancy without previous operation. (3) Two were not pregnant: one had an aortic stricture of 0.8 mm. for 4 days prior to death and the other had an aortic stricture of 1.0 mm. for 9 days prior to death. (4) Two were not pregnant and had no operation prior to death.

Blood pressure was measured in all 32 animals at least every other day according to the method described previously. If the average blood pressure elevation for a specific animal was between 10 and 20 mm. Hg, it was recorded as +; if it was above 20 mm. Hg it was recorded as ++.

The urine was checked for protein in all rabbits at least once; this was done with sulfosalicylic acid. In many, this was done at the time of death on specimens withdrawn from the urinary bladder.

Technique of immunofluorescence. At the time of death renal tissue was quick-frozen on the head of a cryostat. Sections cut at 6 μ were stored at −20° C., stained within 24 to 72 hours with the direct immunofluorescence technique as previously described,[28] and examined on a Leitz Dialux Fluorescence microscope with a mercury lamp, UG 1 excitation filter, and K 510 barrier filter. Specimens were photographed on Anscochrome 200 film.

Fluorescein-conjugated anti–rabbit fibrinogen and anti–rabbit gamma globulin raised in guinea pig were obtained from Behringwerke and showed no staining of control rabbit and human sections of kidney. All slides were examined with coded numbers without knowledge of specific experimental procedure performed and results were recorded and submitted after completion for decoding and analysis. Results of



Fig. 6. Figs. 6 and 7 show sections of kidney from two pregnant rabbits with severe aortic stricture stained with fluoresceinated guinea pig anti–rabbit fibrinogen examined by fluorescence microscopy. Above is shown glomerulus from animal number 5 showing deposits (++) of fibrinogen diffusely scattered in glomerular capillary loops. (Mag. 100 × 4.8.)

staining with antifibrinogen serum were recorded as follows: (1) − when no staining of glomeruli was found; (2) ± weakly positive when only occasional small strands were found in occasional glomerular tufts; (3) + when strands and occasional globular intraluminal masses were found in scattered glomeruli; (4) ++ when many intraluminal masses were found in many or in most glomeruli.

Results. Pregnant animals with severe and prolonged constriction showed deposits of fibrinogen in 14 of 14 animals with weakly positive reactions in only two of 14 (Figs. 6 and 7). In animals with severe but short strictures six of eight were positive, with weakly positive reactions in five of eight and negative reactions in two of eight. In six pregnant animals with sham operations or with no operation, six of six were negative.

In four nonpregnant animals with or without strictures, all were negative for fibrinogen.

In summary, the results with the use of the immunofluorescence technique in 32 animals showed that only in animals with a combination of pregnancy and aortic stricture was fibrinogen demonstrated in glomeruli and that control nonpregnant animals with strictures and pregnant animals with sham operations showed no deposition of fibrinogen in glomeruli.

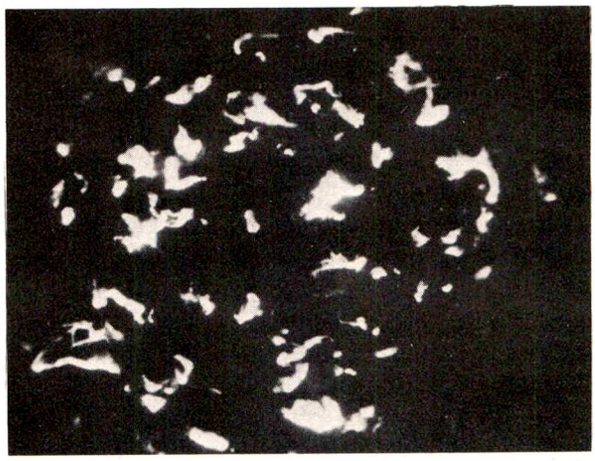


Fig. 7. See legend of Fig. 6. Above is shown glomerulus from animal number 7 showing masses and strands of fibrinogen in glomerular tufts. (Mag. 160 × 4.8.)

Stains for immunoglogulin were negative in all the rabbits.

Comment

Although many—if not most—of the features of experimental rabbit toxemia of pregnancy just described are quite similar to those of human preeclampsia, it seems important to critically review all the findings.

The primary problem with this experimental design is the failure to document the presence of hypertension by a method other than with the Grant-Rothschild capsule. The capsule measures systolic pressure by compression of the central artery of the rabbit's ear and there is subjective bias in deciding at what pressure collapse of the artery has occurred. On the basis of the Grant-Rothschild capsule alone, it may be difficult to accept that hypertension occurred, since the blood pressure in an awake rabbit can vary as much as 10 to 20 mm. Hg from minute to minute. In a sense, the measurement of blood pressure were made blindly, for the degree of arterial constriction was not known until after the animals had been put to death.

Proteinuria was significantly present in more than half of the experimental rabbits and absent in the control animals.

No spontaneous weight gain was observed in the experimental rabbits compared to the normal pregnant rabbits. When fluid and electrolyte retention was elicited indirectly by the injection of normal saline, however, only the animals with experimental toxemia retained the fluid and electrolytes.

The blood pressure and the blood flow below the

aortic constriction were studied following placement of the suture. The results show a marked drop in blood pressure and blood flow when the constriction approached 1 mm. Of course, these results describe only what happens immediately after the suture is placed, and not the continuing effect on the blood circulation. There could be an adaptation of the tonus of the vessels and/or development of a collateral circulation, and these mechanisms could improve the placental circulation in spite of the severe aortic constriction. These figures, however, are similar to those described by Browne and Veall[29] and by Landesman and Knapp,[30] who reported a reduction in the 40 to 50 per cent range of the blood flow to the pregnant uterus of human toxemic patients.

The ultrastructural features of the glomerular capillaries in these animals are similar to those observed in human glomeruli from patients with pre-eclampsia. Endothelial cell swelling and focal fusion of foot processes were common findings. Electron-dense deposits, possibly representing fibrinogen or nonpolymerized fibrin, were noted in 20 per cent of the animals and were located in a subendothelial or submesangial position as in human pre-eclampsia.[22, 31, 32] No definite deposits were seen in the mesangial matrix. No fibrils with the size and periodicity of polymerized fibrin were definitely seen in the glomerular capillaries. In the glomeruli of women with pre-eclampsia electron microscopy often fails to reveal either electron-dense granular deposits or fibrillar fibrin, although immunofluorescence may be positive with antisera to fibrinogen.[28] This discrepancy is best explained by the fact that immunofluorescence is a sensitive immunochemical technique that allows specific identification of proteins and a larger sampling of tissue. Thus a good antifibrinogen antiserum will detect fibrinogen even when present in amounts too small or indistinct (nonpolymerized form) to be discernable by electron microscopy. For example, a comparable situation exists in the glomerulonephritis of Goodpasture's syndrome. In this condition a strong linear fluorescence is often elicited along the glomerular capillary basement membrane with anti-IgG antisera; however, no electron-dense deposits can be detected.[33] The changes of the mesangial cells in these animals are also of interest but are difficult to explain. In human pre-eclampsia mild mesangial hypercellularity is often seen and mesangial cells contain an increased number of mitrochondria, rough endoplasmic reticulum, and electron-dense round bodies—probably phagolysosomes. In the rabbits, the irregular contour of the cells, the density of the cytoplasm, and the prominence of the intracellular fibrils suggest a process of shrinkage or contraction possibly related to arteriolar spasm and to ischemia.

Through the technique of immunofluorescence, material stained by antiserum specific to rabbit fibrinogen is demonstrated to be present in various amounts in the glomeruli of rabbits with experimental toxemia; there was no staining for rabbit gamma globulin. This is evidence that the observed material is fibrin or some derivative of fibrinogen. The controls in this study indicate that deposits of fibrin in glomeruli were absent in the pregnant animals without aortic stricture and in the pregnant animals with a sham operation. Also, the nonpregnant animals without any operation or with a severe aortic stricture did not show the foreign material in the glomerulus. There is no doubt, therefore, that interference with the blood supply to the pregnant uterus creating uteroplacental ischemia of the rabbit near term initiates a process of coagulation and deposition of fibrin products in glomeruli. Of interest is rabbit number 14, with aortic stricture of 7 days' duration, which was killed 9 days after spontaneous delivery. In this case, immunofluorescence was still positive, attesting to the presence of fibrin up to 9 days after delivery. A similar observation was made by other authors[23, 26, 27] who reported the persistence of glomerular lesions a few days after delivery in cases of human toxemia.

In human subjects, even patients with normal pregnancy and no signs of toxemia may show an equivocal increase in glomerular fibrin as compared with normal nonpregnant patients. This was demonstrated by occasional immunofluorescent staining for fibrin.[26, 27] This is not so in the experimental animal, however; in the present study no trace of fibrin was seen in the glomeruli of the control rabbits, whether they were pregnant or not.

The pathophysiology of this fibrin deposition is still under investigation. McKay[15, 17] and others[34, 35] have contributed to the understanding of the relationship between toxemia of pregnancy and disseminated intravascular coagulation. One accepted theory today is that the ischemic placenta releases thromboplastin or other factors which activate the coagulation process. By electron microscopy, fibrin in glomerular of other capillaries can be recognized only when it is present in a highly polymerized form (fibrin polymer) because polymerized fibrin has a fibrillar appearance and a characteristic periodicity. On the other hand, fibrinogen, fibrin monomer, and other intermediate forms appear as granular amophous material of varying electron density. These types of fibrin precursors morphologically can be identified as such only by immunofluorescence.

In conclusion, the clinical manifestations and the morphologic changes obtained in this model of experimental rabbit toxemia are quite similar to those seen in human toxemia and indicate that interference with the blood supply of the pregnant uterus may be an important factor in the pathogenesis of toxemia of pregnancy.

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Cortisol levels in maternal venous and umbilical cord arterial and venous serum at vaginal delivery

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To provide evidence that the high levels of cortisol found in mixed cord serum at spontaneous delivery are derived from the fetus rather than the mother, arterial and venous cord samples were collected separately along with simultaneous maternal venous samples at vaginal delivery following 45 spontaneous and 14 induced labors at term. In the group with spontaneous labor, the difference between umbilical arterial levels of cortisol (78.6 ± 4.1 ng. per milliliter) exceeded venous levels (61.9 ± 4.1 ng. per milliliter) ($P < 0.0001$). In the group with induced labor, the arterial level (58.4 ± 6.9 ng. per milliliter) did not differ significantly from the venous level (50.6 ± 5.9 ng. per milliliter) ($P > 0.05$). In four cases where spontaneous rupture of the membranes occurred prior to induction, values were similar to those of the group with spontaneous labor. The arterial levels of the group with spontaneous labor exceeded those of the group with induced labor ($P < 0.02$). These differences cannot be related to duration of labor, gestational age, or maternal cortisol concentration, none of which differed between the group with electively induced and spontaneous labor. The present data provide further evidence that a rise in fetal cortisol production independent of the mother precedes and is involved in the mechanism initiating the spontaneous onset of human labor.

THE MECHANISM of the initiation of labor in man is unknown. While in some animals, notably sheep, the fetal pituitary-adrenal axis appears clearly to be implicated in the initiation of labor, the only direct evidence in favor of such a mechanism in man has been provided by our previous studies of cortisol and cortisone in mixed cord blood which showed higher cortisol levels in infants born after the spontaneous onset of labor regardless of the mode of delivery.[1] Our recent finding of a rise in amniotic fluid cortisol concentration a week or two prior to the spontaneous onset of labor at term also agrees with this hypothesis.[2] However, while Mati and associates[3] were successful in inducing labor in several postterm women with betamethasone, Liggins[4] has been unable to induce labor at or before term.

To provide further evidence that the high levels of cortisol in cord blood at vaginal delivery are derived from the fetus rather than the mother, arterial and venous cord samples were collected separately in this study, along with simultaneous maternal venous samples. An abstract of this work has appeared.[5]

Material and methods

Immediately following delivery of the infant, a 12 inch segment of umbilical cord was clamped at the placental and fetal ends before delivery of the placenta. The umbilical arteries and vein were identified, and blood was withdrawn first from the arteries, then from the vein. A maternal peripheral venous blood sample was also drawn at the time of delivery. After centrifuging, the serum was separated and frozen until analyzed.

Cortisol was determined by a highly specific nonchromatographic radiotransinassay described recently.[6]

Sixty-three mother-infant pairs were included in the study. Of these, 45 were delivered vaginally after

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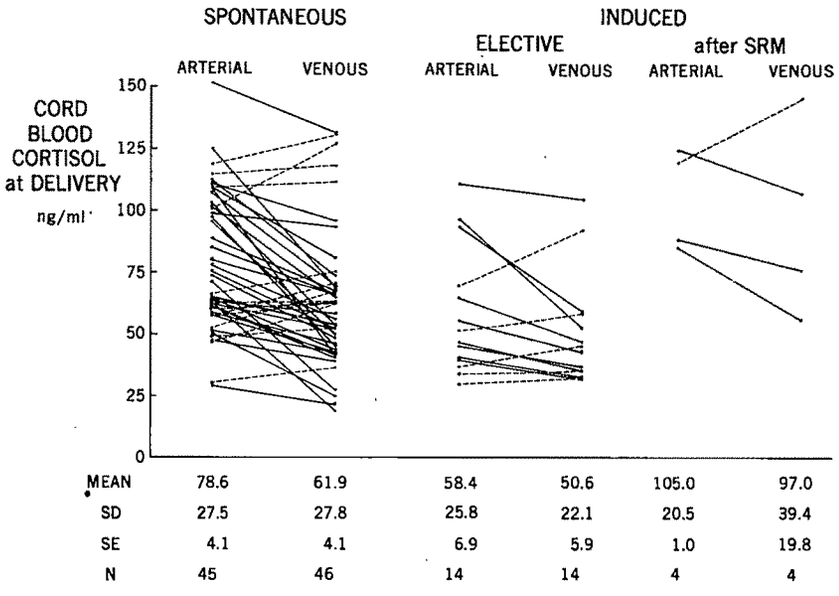


Fig. 1. Cord serum cortisol levels at vaginal delivery.

Table I. Correlations

Onset of labor	*Sample*	*No. of pairs*	*r*	*t*	*p*
Spontaneous	Cord arterial vs. cord venous	44	0.69	6.2	<0.001
Spontaneous	Cord arterial vs. maternal venous	42	0.19	1.2	N.S.
Spontaneous	Cord venous vs. maternal venous	42	0.50	3.3	<0.01
Induced	Cord arterial vs. cord venous	14	0.77	4.2	<0.01
Induced	Cord arterial vs. maternal venous	14	0.43	1.6	N.S.
Induced	Cord venous vs. maternal venous	14	0.69	3.3	<0.01

N.S. = Not significant.

spontaneous onset of labor, 14 had labor electively induced with intravenous oxytocin administered by a continuous-infusion pump at a rate of 1 to 10 MU. per minute and four had induction of labor within 24 hours of spontaneous rupture of the membranes (SRM). All patients were at term (> 36 weeks). The mean gestational ages were: for the spontaneous group, 40.0 weeks (36 ½ to 42 ½); for the group with elective induction, 40.2 weeks (38 to 42); and for the group with induction after SRM, 39 weeks (37 to 40).

Results

The duration of spontaneous labor (8.95 hours ± 0.89 standard error) did not differ ($P > 0.05$) from that of induced labor (6.7 ± 1.18 hours).

Maternal levels of cortisol in the group with induced labor (520 ± 50 ng. per milliliter) were slightly higher than those in the group with spontaneous labor (457 ± ng. per milliliter), but this difference was not significant ($P > 0.05$).

Umbilical arterial and venous levels for the various groups are shown in Fig. 1. In the group with spontaneous labor, cord arterial levels of cortisol (78.6 ± 4.1 ng. per milliliter) exceeded cord venous levels (61.9 ± 4.1 ng. per milliliter) ($P < 0.01$), while in the group with induced labor the arterial level (58.4 ± 6.9 ng. per milliliter) did not differ ($P > 0.05$) from the venous level (50.6 ± 5.9 ng. per milliliter). When the arterial-venous differences were paired, the significance of the difference in the group with spontaneous labor was even more marked ($P < 0.0001$) but that of the group with induced labor was unchanged. Of the four with spontaneous rupture of the membranes prior to induction of labor three had higher arterial than venous levels.

When the arterial levels of the group with spontaneous labor were compared with those of the group with induced labor, the level of the former was higher ($P < 0.02$) than in the latter without SRM but was similar to the level in the group with prior rupture of the membranes.

The correlation coefficients for various groups are

shown in Table I. The correlation between the cord arterial and venous levels in the groups with both spontaneous and induced labor was significant as was the relation between maternal and cord venous levels. However, no good correlation was present between cord arterial and maternal venous levels.

Comment

Umbilical arterial and venous cortisol levels have rarely been compared. In 6 midterm fetuses, we found that the arterial cortisol levels exceeded the venous levels ($P < 0.01$).[7] Turner and colleagues[8] found that the arterial values for six normal vaginal deliveries (73 ± 45 S.D. ng/ml) exceeded the venous levels (49 ± 26 S.D. ng/ml) $P < 0.05$) and attributed this to the stress of delivery. Smith and Shearman[9, 10] found higher venous than arterial levels at delivery whether vaginal or by cesarean section. However, their method measured cortisone, 17-hydroxprogesterone, and probably some progesterone as well as cortisol so that their results are impossible to interpret.

Since the maternal venous cortisol level is known to rise considerably during labor,[11] it might be expected that a rise in cord blood cortisol might well be due to maternal cortisol crossing the placenta as suggested by Pokoly.[12] If this is true, then the venous cord cortisol level should be higher than the arterial level. However, in the group with spontaneous labor, the arterial level clearly exceeded the venous level so that one must accept that more cortisol is coming from the fetus than is being transferred to it. If we do accept this, then might not the high fetal level be due to the "stress" of labor itself?

That this high cortisol level is due rather to a rise in fetal cortisol occurring prior to the spontaneous onset of labor is suggested by the significantly higher arterial levels in the group with spontaneous labor and in the group with induced labor and SRM than in the group with electively induced labor. This group with induced labor and SRM has been considered separately since it is known that at term 80 per cent of patients go into spontaneous labor within 24 hours of rupture of the membranes while 90 per cent are delivered within 48 hours; so presumably these patients were about to go into spontaneous labor. These differences cannot be related to duration of labor, gestational age, or maternal cortisol concentration, none of which differed between the groups with electively induced and spontaneous labor.

The values in the group with electively induced labor are higher than those observed in our original study of mixed cord blood (24 ± 4.5 ng. per milliliter). This is probably due to the fact that patients in the first group had labor induced mainly at 38 to 39 weeks rather than at 40 weeks, as here. Such an interpretation is also suggested by the high levels seen following induction after SRM.

Therefore, the present data provide further evidence that a rise in fetal cortisol production independent of the mother precedes the spontaneous onset of human labor and suggest that this change is associated with the initiating mechanism.

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Association between maternal bleeding during gestation and congenital anomalies in the offspring

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The incidence of maternal bleeding complicating the pregnancies of 326 children examined at the Jerusalem Community Center for Child and Family Development is surveyed. A history of bleeding during pregnancy was more frequent in children with congenital anomalies of the central nervous system (and/or psychomotor retardation) (33 per cent) and in children with congenital anomalies of other systems (29 per cent) than in control children (12 per cent). The implications of these findings are discussed in relation to placental pathophysiology and the pathogenesis of threatened abortion.

HYPOXIA is known to influence fetal development sufficiently to induce anomalies in various animal species.[7, 10] Human intrauterine growth retardation in the later months of gestation[5] has been attributed to hypoxia but no strong evidence has emerged that it is teratogenic.

Various investigators have reported prospective and retrospective studies in which they attempted to establish a correlation between pregnancy bleeding and poor fetal outcome.[6, 14, 15, 17, 18, 21]

In most of the prospective studies no such direct relation could be demonstrated, possibly because of the low total number of anomalies. Where larger numbers were involved, as in the 8,000 pregnancies followed up from the first trimester by Rougette and associates,[19] a greater incidence of genital bleeding during the first trimester was reported by mothers of children born with defects (24 per cent) than by the mothers of control infants (14 per cent).

The retrospective studies have usually yielded a greater frequency of anomalies, and most investigators have agreed there is a positive relation between the complications of pregnancy, including bleeding, and congenital anomalies or small and premature infants.[1, 14, 21] Most of these studies were based on data obtained retrospectively from routine hospital or clinic files and the incidence of bleeding was undoubtedly underestimated. The same reservation may well apply to the recorded incidence of anomalies, since the figures were based mainly upon neonatal records, so that many anomalies probably remained undiagnosed. This would be particularly true for mental retardation, so that, not unexpectedly, the literature on the possible relation between complications of pregnancy and mental retardation is sparse.[4, 17]

In the Jerusalem Community Center for Child and Family Development, children from the age of 1 month with or suspected of having congenital abnormality or psychomotor retardation undergo separate comprehensive examinations by a psychologist and a developmental pediatrician. Before these examinations, each mother is interviewed and a detailed questionnaire about various aspects of her pregnancy and previous obstetric history is completed.

We report below our findings with reference to gestational bleeding in mothers of 326 children examined during the last 3 years in our Center.

Materials and methods

A total of 326 children between the ages of 6 months and 5 years who underwent comprehensive interdisciplinary assessment at our Center between 1971 and

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Table I. Occurrence of maternal bleeding during pregnancy in 326 infants examined*

	Congenital anomalies excluding CNS			*Congenital CNS anomalies including chromosomal defects*			*Controls (no anomalies, normal development)*		
	Total	*Without bleeding*	*With bleeding*	*Total*	*Without bleeding*	*With bleeding*	*Total*	*Without bleeding*	*With bleeding*
No. of infants	63	45	18	110	74	36	153	134	19
Percentage		71	29		67	33		88	12

*No statistically significant difference between non-CNS and CNS congenital anomalies. Highly significant difference between congenital anomalies and controls: $p < 0.01$.

1973 were referred from various clinics and newborn departments in the city of Jerusalem because of diagnosed or suspected congenital abnormality or psychomotor retardation. Examination showed many of them to be normal and we therefore decided to use the normal children as controls for this study.

Before the children were examined, each mother was interviewed by a developmental nurse and a social worker, members of our interdisciplinary subunits, and the answers were recorded on a standard form. Various details of the pregnancy and previous obstetric and medical history were recorded, with special reference to potential teratogenic factors such as bleeding, drug ingestion, or irradiation during pregnancy.

The children then underwent a developmental examination by a clinical psychologist using the Bayley[2] Scales of Mental and Motor Development, standardized for Israeli populations by Kohen-Raz.[11, 12] For the purpose of a more refined clinical diagnosis the original Bayley Scales were broken down into subscales of mental and motor functions based on a Scalogram Analysis[11] such as Mental Scales of Manipulation, Eye-Hand Coordination, Object Relation, Imitation, Comprehension, and Active Social Contact, Verbalization, as well as Motor Scales of Static Coordination, Body Coordination, Arm Coordination, Fine Coordination, and Walking.

Following this a full neurologic and pediatric examination was completed.

Most of the children were so examined on at least two occasions. A small number found to be normal on initial examination were not examined a second time.

For the purpose of this study the children were divided into three groups: (1) 153 children (control subjects) with normal development and without congenital abnormalities; (2) 63 children with congenital anomalies (excluding children with central nervous system anomalies) but with normal mental development; (3) 110 children with congenital abnormalities of the central nervous system (CNS) or with psychomotor retardation (including 14 cases of Down's syndrome and 8 cases of CNS anomalies without mental retardation).

CNS damage and significant motor retardation on the Bayley Scales was qualitatively manifest in conspicuous retardation of Fine Motor Coordination, poor Eye-Hand Coordination, and lack of Object Relation as well as in a longitudinal decrease of developmental quotients, indicating tendencies of stagnation and inhibited growth.[13] Quantitatively, psychomotor retardation and suspect CNS damage was operationally defined as a score equal to or less than 70 on the mental scales on two consecutive tests, or a score of 50 or less in one examination when the infant was over the age of 1 year.

We wish to report here our findings on the incidence of vaginal bleeding during the pregnancies with those infants. The mothers were asked whether bleeding or spotting occurred during gestation, if so, how long after the last menstrual period it first appeared, and its duration and intensity. Women with spotting only were considered "positive." Known cases of placenta previa were omitted. The placentas were not examined.

Results

The mothers from the three groups were of similar socioeconomic class, ethnic origin, and age.

A significantly increased incidence of bleeding was found in both congenital anomaly groups (Table I). In both these groups the incidence of bleeding was over 2½ times that in the control group ($P < 0.01$). There was, however, no significant difference between groups of CNS anomalies vs. non-CNS congenital anomalies, and there was no difference in the incidence of bleeding between the sexes.

Table II shows the incidence of bleeding in the groups with congenital anomalies when 21 cases of known hereditary etiology (10 from the group of congenital CNS anomalies and 11 from the group of non-CNS congenital anomalies (Table III) were excluded. In both groups the incidence of bleeding rose to more than one third of the total cases, being almost three times the incidence of bleeding in the control group.

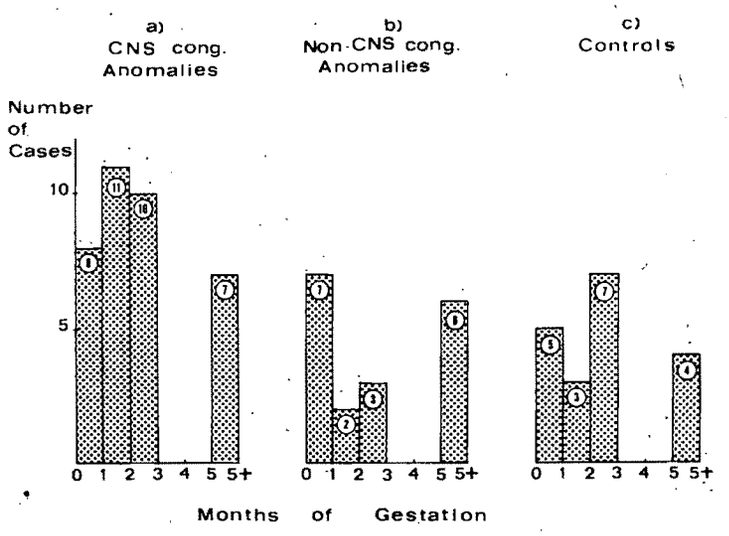


Fig. 1. Distribution of maternal bleeding according to month of gestation.

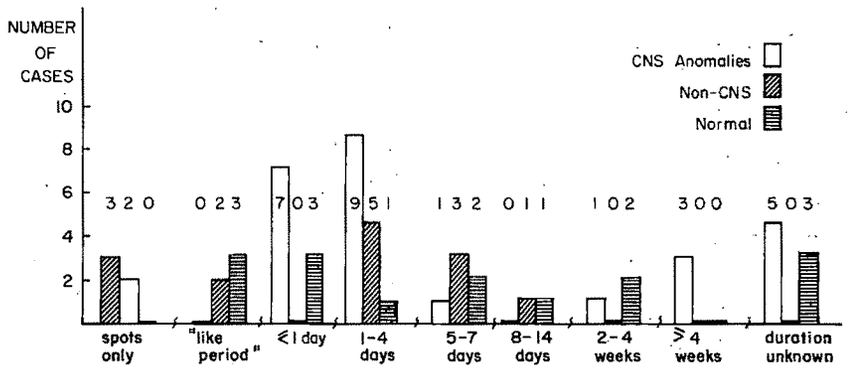


Fig. 2. Duration and nature of bleeding starting in the first trimester (57 cases). "Like period": Regular bleeding at the time of expected period described by the patient as such.

Fig. 1 represents a breakdown of the incidence of bleeding according to the month of gestation when bleeding first appeared. It will be seen that most examples of bleeding started in the first trimester of pregnancy (gestational age recorded according to the date of the last menstrual period); in a minority of cases bleeding started after the fifth month; no instance of bleeding was found to have started during the fourth and fifth months.

Fig. 2 describes the duration and nature of vaginal bleeding starting in the first trimester. There were five cases with spots only and five cases with bleeding at the time of the expected period. In 16 of 29 cases of CNS anomalies, duration of bleeding was 1 to 4 days.

Table IV indicates the percentage incidence of bleeding in the first trimester in all groups and Table V lists the conditions associated with first-trimester bleeding. When diseases of known hereditary etiology are again excluded (Table VI), the incidence of bleeding starting in the first trimester rises to 25 per cent in the group with congenital non-CNS anomalies and to 28 per cent in the group with CNS anomalies or mental and psychomotor retardation, as opposed to only 10 per cent in the controls (Table IV).

Comment

There is insufficient information concerning the possible deleterious effects of early vaginal bleeding upon the human fetus. We can presume that bleeding originating in the placental area may be associated with partial separation of the placenta[9] which can be manifested at a later stage of pregnancy in the form of partial or complete abruptio placentae. This type of disturbance could undoubtedly interfere with some placental function, possibly to the extent of leading to fetal hypoxia and/or malnutrition.[3, 5]

Table II. Occurrence of maternal bleeding in 152 infants with congenital anomalies excluding disorders with known hereditary pattern

	Congenital anomalies non-CNS			*CNS anomalies*		
	Total	*Without bleeding*	*With bleeding*	*Total*	*Without bleeding*	*With bleeding*
No. of patients	52	34	18	100	65	35
Percentage		66	34		65	35

Table III. Diagnoses of 21 infants with congenital anomalies of known hereditary pattern

	No.
With first-trimester bleeding:	
Cleidocranial dysostosis (+ PMR)	1
Without first-trimester bleeding:	
Marinescu-Sjögren syndrome	2
Von Gierke's disease	2
Riley-Day syndrome	1
Von Recklinghausen's disease	1
Tay-Sachs disease	1
Ellis-van Creveld syndrome	1
Weidermann Beckwith syndrome	1
Thalassemia	1
Achondroplasia	1
Windmill syndrome	2
Albinism	1
Familial dwarfism	2
Familial deafness	2
Familial cataracts	1

Table IV. Occurrence of maternal bleeding in first trimester of pregnancy in 326 infants examined*

	Non-CNS anomalies		*CNS anomalies*		*Controls*	
	Total	*Bleeders*	*Total*	*Bleeders*	*Total*	*Bleeders*
No. of infants	63	13	110	29	153	15
Percentage		21		26		10

*No statistically significant differences between non-CNS and CNS congenital anomalies. Highly significant difference between congenital anomalies and controls: $P < 0.01$.

In the collaborative perinatal study of the National Institute of Neurological Diseases and Stroke[15] attention was given to the problem of vaginal bleeding during pregnancy. The authors found not only that bleeding increases the risk of fetal and neonatal death or of low birth weight, but that neurologic impairment at 1 year of age was found in a higher percentage in infants whose mothers had bleeding during the second trimester than in control infants. In cases of partial abruptio placentae, very similar results were found regarding birth weight, neonatal death, and neurologic impairment at 1 year of age.

Table V. Diagnoses of 42 infants with congenital anomalies associated with bleeding in the first trimester

	No.	
Psychomotor retardation	20	
Psychomotor retardation associated with non-CNS anomaly	3	(hernia + CDH) (harelip + cleft palate) (cleidocranial dysostosis)
CNS anomaly without psychomotor retardation	2	(petit mal, meningomyelocele)
Mongolism	4	
Non-CNS anomalies:		
Congenital heart disease	6	
Renal anomalies (polycystic kidney, renal dysostosis)	2	
Hare lip + cleft palate	1	
Hernia	1	
Polydactyly	1	
Hypospadias, cryptorchidism, club foot	1	
Congenital cirrhosis	1	

Table VI. Occurrence of maternal bleeding in the first trimester of pregnancy in 152 infants with congenital anomalies, excluding disorders with known hereditary pattern*

	Congenital anomalies (non-CNS)		*CNS anomalies*	
	Total	*Bleeders*	*Total*	*Bleeders*
Total number of infants	52	13	100	28
Percentage		25		28

*The difference between the groups is not statistically significant.

It is not clear, however, whether placental injury earlier in gestation can be responsible for congenital anomalies. One of the first placental functions to be affected by placental damage is oxygen transfer[21] and fetal nutrition.[3, 5] Severe hypoxia and/or malnutrition will result in fetal death, and less severe damage may interfere with fetal growth, but, during the period of organogenesis, one can only speculate upon such teratogenic effects of hypoxia or malnutrition.[8] Certainly there is abundant evidence that gestational hypoxia is teratogenic in some species.[7, 10] Although direct evidence is lacking, our results may suggest a similar effect upon the human embryo.

In favor of the hypothesis that bleeding early in pregnancy is a cause rather than a mere accompaniment might be the fact that in the group of anomalies

with a known hereditary pattern the incidence of bleeding was very low (one case in a total of 21 patients—Table III). On the other hand, in our series of Down's syndrome, four out of 14 mothers had genital bleeding. In these cases the bleeding is clearly not the cause of the defect and this finding may point to an association of chromosomal aberration to pregnancy bleeding, although the numbers are too small to be conclusive. Similarly, Rougette and associates[19] found a high incidence of gestational bleeding in genetically determined anomalies. The possibility cannot be excluded that an association similar to that found between genetically determined anomalies and bleeding, which cannot be related on the basis of cause and effect, may occur in other anomalies.

Although in many cases the causes for vaginal bleeding during gestation (threatened abortion) are unknown, sometimes abnormal placentation, uterine anomalies, or infections are encountered as possible causes.[3] Of particular importance are inflammatory lesions of the decidua or placenta. During the rubella epidemic in Israel in 1972, fetuses and placentas from women whose pregnancies were interrupted due to gestational rubella were studied. It was found that in 50 per cent the placentas showed various inflammatory lesions.[16] From eight infants with the congenital rubella syndrome examined in our Developmental Center (not included in this study), there was a positive history of genital bleeding in three. Although direct evidence is lacking, we might suspect that intrauterine infection can be responsible for a gestational bleeding in o

Our study, like all ret the criticism that mother more likely to recall abno worth stressing, however, Center because of suspec psychomotor retardation each case was carried ou examination was known; factor was insignificant ir

The population exam selected, and the numbe conclusions to be drawn effect of bleeding in ea percentage of bleeding suffered from congenital clearly significant of at le two. Extensive prospect establish the incidence c psychomotor retardation tion, which may be im intensive treatment of th

A proper experimenta effects of gestational blee is lacking. Reduction of o of uterine arteries[5] in pr being the appropriate co els.

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an oral contraceptive are many. Some are hers and some are yours.

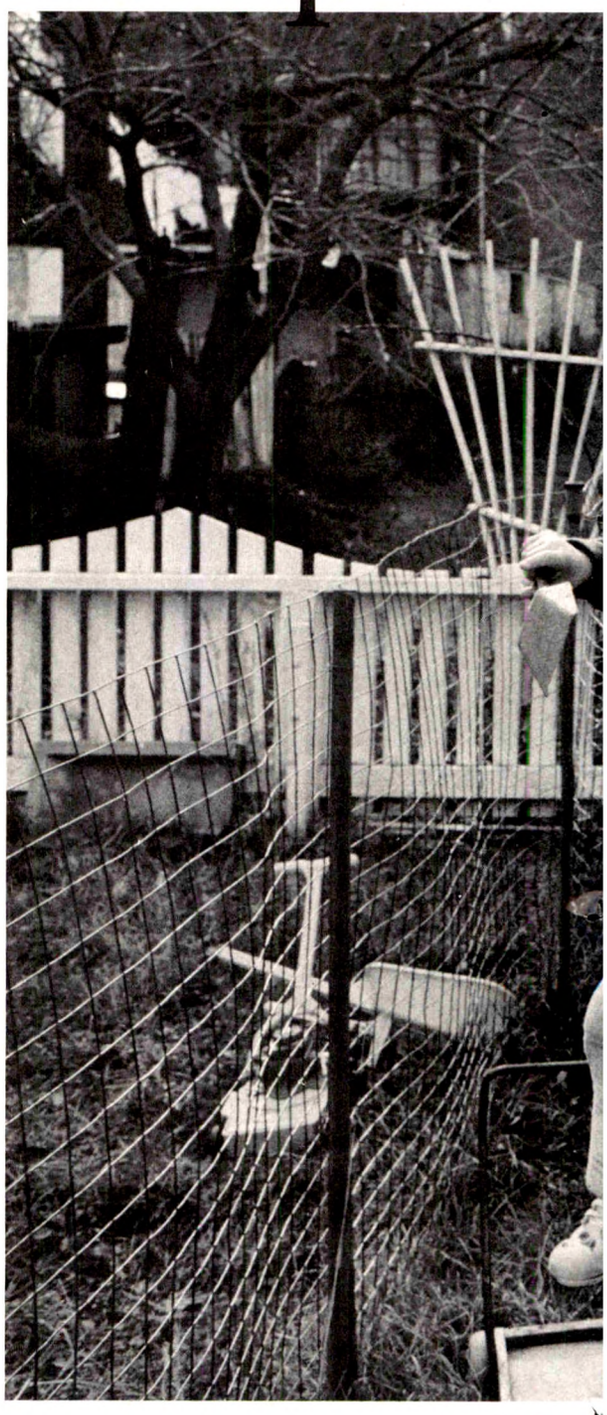
In the main, her reasons for rejection, often highly subjective, are based on side effects—and side effects are only partially predictable from history and examination.

The ultimate test, then, of oral contraceptive acceptability must be what happens when she takes it since both your judgment and hers will be based on...

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One important reason for patient satisfaction: Usually no G.I. discomfort

ZORANE 1/50 is a highly effective and generally well-tolerated oral contraceptive—providing the estrogen content and progestin:estrogen ratio that appears to be highly acceptable to the majority of women.

From clinical studies: In a study population of 1156 women, 25,983 cycles, the incidence of nausea and vomiting was 3.3%. Actual patient dropout rate* due to nausea and vomiting was 2.9% (33 subjects.) Overall patient dropout rate was 14.4% due to all adverse reactions. Serious reactions, as well as minor adverse reactions, have been reported following the use of all oral contraceptives. Before prescribing, the complete product information should be consulted for *special note, contraindications, warnings, precautions* and *adverse reactions*. Thereafter, the physician should remain alert to the earliest manifestations of any serious disorder. Should any occur or be suspected, the oral contraceptive should be immediately discontinued.

The woman profiled here is an actual ZORANE 1/50 user. However, not all women can be expected to have the same tolerance for side effects, should any occur. (Data on file, Lederle Laboratories)

*Dropout rates are based on total patient population (1,156); incidence reflects only those patients who completed one or more cycles (956).

Zorane® 1/50

+ Norethindrone Acetate, 1 mg and Ethinyl Estradiol, 50 mcg
Oral Contraceptive Tablets

Progestin/Estrogen content balanced for

See last page of this advertisement for brief summary of prescribing information.

with



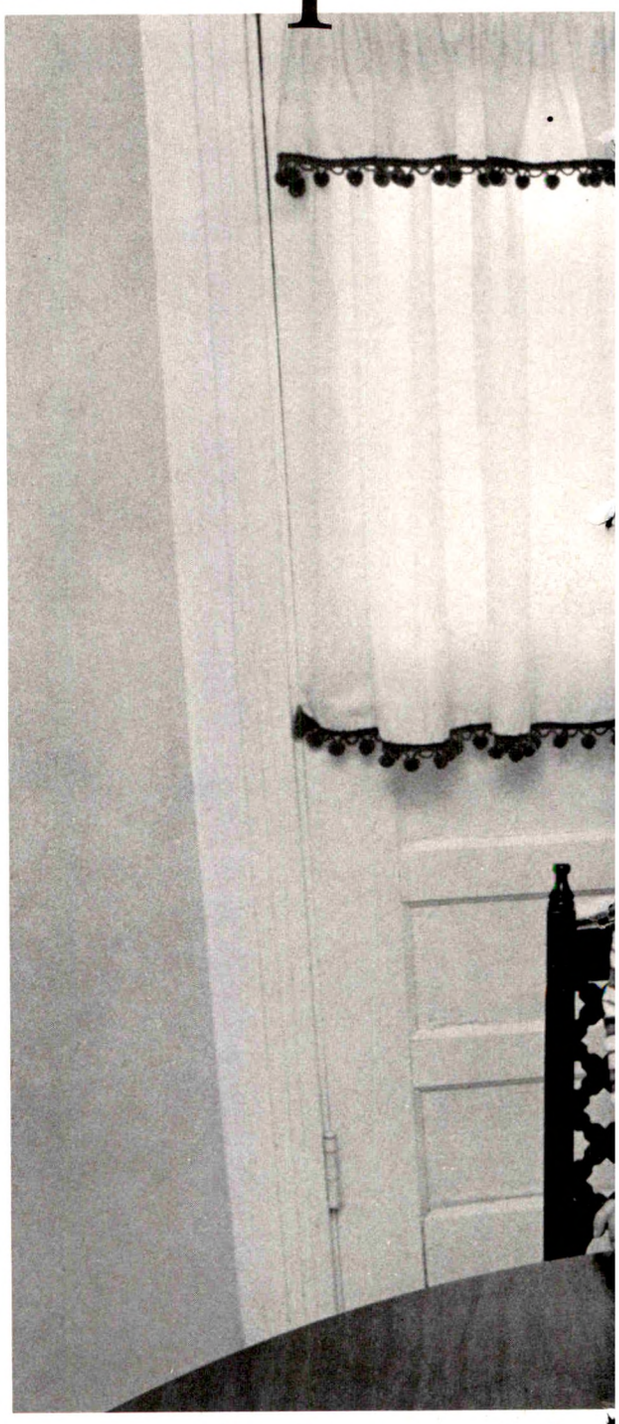
Judy Barrett, Housewife
Pittston, Pennsylvania
Age 22, married 6 years, 2 children
Cycle experience with Zorane 1/50: 8

the majority

"I don't seem to gain much weight with the pill and I don't get sick. I take it at night just before I go to bed... then I sleep right through. And I don't get sick."

From real life...

A Woman satisfied her oral contraceptive



Among the reasons patients remain satisfied: Withdrawal bleeding is usually regular and there is usually no undue discomfort

ZORANE 1/50 is a highly effective and generally well-tolerated oral contraceptive—providing the estrogen content and progestin:estrogen ratio that appears to be highly acceptable to the majority of women.

From clinical studies: In a study population of 1156 women, 25,983 cycles, the incidence was 2.2% for amenorrhea and 2.0% for dysmenorrhea. Actual patient dropout rate* was 1.1% (13 subjects) for amenorrhea and 0.2% (2 subjects) for dysmenorrhea. Overall patient dropout rate was 14.4% due to all adverse reactions. Serious reactions, as well as minor adverse reactions, have been reported following the use of all oral contraceptives. Before prescribing, the complete product information should be consulted for *special note, contraindications, warnings, precautions* and *adverse reactions*. Thereafter, the physician should remain alert to the earliest manifestations of any serious disorder. Should any occur or be suspected, the oral contraceptive should be immediately discontinued.

The woman profiled here is an actual ZORANE 1/50 user. However, not all women can be expected to have the same tolerance for side effects, should any occur. (Data on file, Lederle Laboratories)

*Dropout rates are based on total patient population (1,156); incidence reflects only those patients who completed one or more cycles (956).

Zorane® 1/50

+ Norethindrone Acetate, 1 mg and Ethinyl Estradiol, 50 mcg

Oral Contraceptive Tablets

Progestin/Estrogen content balanced for

See last page of this advertisement for brief summary of prescribing information.

with



Mrs. Linda McKinnon, Housewife
Nederland, Texas
Age 25, married 7 years, 2 children
Cycle experience with Zorane 1/50: 16

the majority

"You always know when you're going to start…I love knowing that… you can always plan…I think it's great. And, not having any cramps, too, because I used to have cramps."

From real life...

A Woman satisfied her oral contraceptive



One reason for long-term patient satisfaction: Weight gain usually not a problem

ZORANE 1/50 is a highly effective and generally well-tolerated oral contraceptive—providing the estrogen content and progestin:estrogen ratio that appears to be highly acceptable to the majority of women.

From clinical studies: In a study population of 1156 women, 25,983 cycles, the incidence of weight gain was 0.4%. Actual patient dropout rate* due to weight gain was 2.0% (23 subjects). Overall patient dropout rate was 14.4% due to all adverse reactions. Serious reactions, as well as minor adverse reactions, have been reported following the use of all oral contraceptives. Before, prescribing, the complete product information should be consulted for *special note, contraindications, warnings, precautions* and *adverse reactions*. Thereafter, the physician should remain alert to the earliest manifestations of any serious disorder. Should any occur or be suspected, the oral contraceptive should be immediately discontinued.

The woman profiled here is an actual ZORANE 1/50 user. However, not all women can be expected to have the same tolerance for side effects, should any occur. (Data on file, Lederle Laboratories)

*Dropout rates are based on total patient population (1,156); incidence reflects only those patients who completed one or more cycles (956).

Zorane® 1/50

+Norethindrone Acetate, 1 mg and Ethinyl Estradiol, 50 mcg

Oral Contraceptive Tablets

Progestin/Estrogen content balanced for

See last page of this advertisement for brief summary of prescribing information.

with



Alice Eichenberger, High School
Business Teacher, Louisville, Kentucky
Age 28, married 7 years, no children
Cycle experience with Zorane 1/50: 13

the majority

"I've fluctuated between 115 and 120...and have had no appreciable weight gain...since I've been on Zorane."

Zorane® 1/50

Progestin/Estrogen content balanced for the majority

Norethindrone Acetate, 1 mg and Ethinyl Estradiol, 50 mcg

Oral Contraceptive Tablets

Also available from Lederle

Zorane 1.5/30 Norethindrone Acetate, 1.5 mg, and Ethinyl Estradiol, 30 mcg.

Zorane 1/20 Norethindrone Acetate, 1 mg, and Ethinyl Estradiol, 20 mcg.

Special Note: Oral contraceptives have been marketed in the United States since 1960. Reported pregnancy rates vary from product to product. The effectiveness of sequential products appears to be somewhat lower than that of combination products. Both types provide almost completely effective contraception. The dropout rate for medical reasons, as observed in clinical trials, with ZORANE 1.5/30 appears to be the same as that of higher dose combination products. The dropout rate due to irregular bleeding was somewhat higher, but dropouts were equally split between irregular bleeding and other medical reasons attributable to the drug. (ZORANE 1/20 only: The effectiveness is somewhat lower than that of the higher dose combination products. Both provide almost completely effective contraception. Dropout rate for medical reasons appears to be somewhat higher than with higher dose combination products, due particularly to irregular bleeding.) An increased risk of thromboembolic disease associated with use of hormonal contraceptives has now been shown in studies conducted in both Great Britain and the United States. Other risks, such as those of elevated blood pressure, liver disease, reduced tolerance to carbohydrates, have not been quantitated with precision. Long-term administration of both natural and synthetic estrogens in subprimate animal species in multiples of the human dose increases the frequency for some animal carcinomas. These data cannot be transposed directly to man. Possible carcinogenicity due to the estrogens can neither be affirmed nor refuted at this time. Close clinical surveillance of all women taking oral contraceptives must be continued.

Indications: For control of conception.

Contraindications: Thrombophlebitis, thromboembolic disorders, cerebral apoplexy, or a past history of these conditions; markedly impaired liver function; known or suspected carcinoma of the breast; known or suspected estrogen-dependent neoplasia; undiagnosed abnormal genital bleeding; pregnancy.

Warnings: If early signs of thrombotic disorders (thrombophlebitis, cerebrovascular disorders, pulmonary embolism, retinal thrombosis) occur or are suspected, discontinue use of drug immediately. (Studies indicated a higher risk of these conditions with use of these drugs, not persisting after drug stoppage and not enhanced by long-term use.[1-4]) If sudden partial or complete loss of vision occurs, or if there is sudden onset of proptosis, diplopia or migraine; or if examination reveals papilledema or retinal vascular lesions, discontinue medication. Safety of use in pregnancy has not been established, therefore rule out pregnancy in any patient who has missed two consecutive periods before continuing contraceptive regimen; if prescribed schedule has not been adhered to, consider possibility of pregnancy with first missed period. A small fraction of hormonal agents has been identified in the milk of mothers receiving these drugs; long-range effect to the nursing infant has not yet been determined.

Precautions: Pretreatment and periodic physical examinations of breasts and pelvic organs should include Papanicolaou smear. (Estrogens have produced tumors, some malignant, in 5 species of subprimate animals.) If endocrine and liver function tests are abnormal in patients taking these drugs, repeat tests after drug withdrawal for 2 months. With use of these drugs, pre-existing uterine fibromyomata may increase in size; and some conditions (*i.e.*, epilepsy, migraine, asthma, cardiac or renal dysfunction) may be influenced by fluid retention; observe carefully for such. With use of ZORANE 1.5/30 or ZORANE 1/20, alteration in menstrual patterns may be induced. Since the amount and duration of flow and cycle length may vary, this must be considered in attempting to determine if cause of bleeding is organic. (ZORANE 1/50 only: In breakthrough bleeding or all cases of irregular bleeding per vaginam, keep in mind nonfunctional causes and take adequate diagnostic measures.) With history of psychic depression, discontinue drug if depression recurs to a serious degree. Possible influence of prolonged ZORANE therapy on pituitary, ovarian, adrenal, hepatic or uterine function awaits further study. A decrease in glucose tolerance has been seen in some patients on oral contraceptives; carefully observe diabetics on such therapy. Patient age is no absolute limiting factor, although these drugs may mask onset of climacteric. The pathologist should be advised of ZORANE therapy when relevant specimens are submitted. Susceptible women may have an increase in blood pressure following use of contraceptive steroids.

Adverse Reactions Observed in Patients Receiving Contraceptives: Statistically significant association demonstrated between use of oral contraceptives and: thrombophlebitis, pulmonary embolism, cerebral thrombosis. Suggestive evidence, neither confirmed nor refuted, between use and neuro-ocular lesions (*e.g.*, retinal thrombosis, optic neuritis). Known to occur: nausea, vomiting, G.I. symptoms (abdominal cramps, bloating), breakthrough bleeding, spotting, change in menstrual flow, amenorrhea during and after treatment, edema, chloasma or melasma, breast changes (tenderness, enlargement, secretion), weight change (increase or decrease), changes in cervical erosion and secretions, suppression of lactation when given immediately post-partum, cholestatic jaundice, migraine, allergic rash, rise in blood pressure in susceptible women, mental depression. Reported association with use, neither confirmed nor refuted: anovulation post treatment, premenstrual-like syndrome, changes in libido, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, fatigue, backache, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, itching. *May Alter Laboratory Results in:* hepatic function (increased sulfobromophthalein retention and other tests), coagulation tests (increase in prothrombin, Factors VII, VIII, IX, X), thyroid function (increase in PBI_1, and butanol extractable protein bound iodine, and decrease in T^3 uptake values), metyrapone test, pregnanediol determination.

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FETUS, PLACENTA, AND NEWBORN

A comparison of two glucocorticoid regimens for acceleration of fetal lung maturation in premature labor

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WILLIAM H. SCRAGG, M.D., F.A.C.O.G.
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This study compares fetal corticoid response from conventional dose (12.0 mg.) intramuscular betamethasone to large dose (1,000 mg.) intravenous cortisol administered to women in premature labor for acceleration of fetal lung maturity. To compare these two regimens, 14 women selected at random were treated in groups of seven with either cortisol or betamethasone. Peripheral levels of unconjugated estriol were measured by specific radioimmunoassay prior to the corticoid dose and at 1, 4, 8, and 12 hours following the dose. The rate of corticoid delivery to the fetal hypothalamic-adrenal axis was estimated by the per cent suppression of unconjugated estriol at each post-treatment interval. Least-squares regression lines fitted ($P < 0.01$) for each regimen were compared for time saved (Δt) when cortisol was used. Mean Δt (1, 4, 8, and 12 hours) was 9.0 ± 0.2 S.E.M. hours. It is concluded that: (1) Intravenous cortisol delivers a fetal corticoid effect that is significantly more rapid in onset and more profound in magnitude than does intramuscular betamethasone and that (2) the cortisol regimen is probably better suited to the acceleration of fetal lung maturation in premature labor when time is short and rapid action is essential.

SEVERAL studies of glucocorticoid effect on fetal lung maturation have utilized varying regimens of intramuscular betamethasone,[1, 2] a synthetic, high-potency, long-acting analogue of cortisol. The efficiency and speed at which this drug crosses the placenta and exerts its pharmacologic effect at reported dose schedules has not been studied. In addition, there has as yet been no published investigation into potentially quicker acting corticosteroid regimens applicable to accelerating fetal lung maturation during premature labor when time is short and rapid action is essential.

With the use of suppression of unconjugated maternal serum estriol (E_3)[3] levels as an indicator of fetal hypothalamic-adrenal axis response,[4] the following

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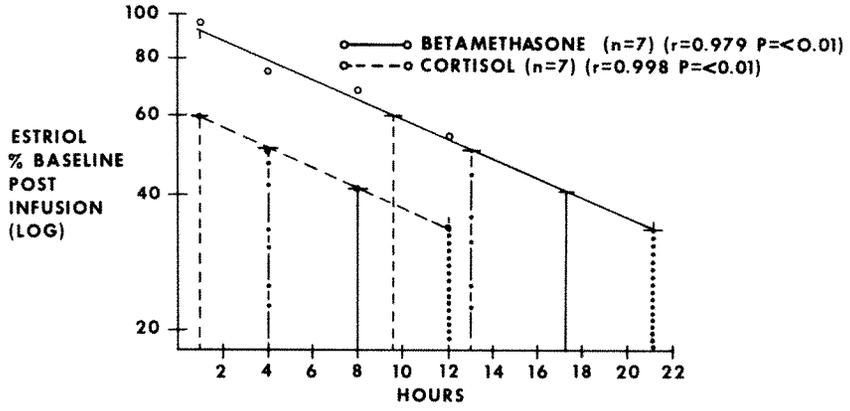


Fig. 1. Time difference (Δt) in estriol suppression between two corticoid regimens. Regimen C produces an equivalent pharmacologic effect 9.0 ± 0.2 S.E.M. hours (Δt) more rapidly than does Regimen B. Δt:•First hour, 8.6; fourth hour, 9.0; eighth hour, 9.2; twelfth hour, 9.3.

Table I. Suppression (per cent baseline ± S.E.M.) obtained for each of the post-treatment intervals

Treatment	*1 hour*	*4 hours*	*8 hours*	*12 hours*
Betamethasone	96 ± 2	75 ± 11	68 ± 8	54 ± 7
Cortisol	60 ± 2	50 ± 5	41 ± 7	34 ± 6
Unpaired t test	$P < 0.001$	$0.05 < P << 0.1$	$P < 0.05$	$P < 0.05$

studies were designed to characterize the rate of transplacental delivery of corticoid effect afforded by a standard (12.0 mg.) dose of intramuscular betamethasone and to determine if a more immediate and profound corticoid effect could be produced by a larger dose (1,000 mg.) of intravenous cortisol.*

Materials and methods

Fourteen women admitted in premature labor between 28 and 34 weeks of estimated gestational age and being otherwise without complications were begun on intravenous alcohol[5] to delay, or, if possible, halt progress of labor. By random selection, seven women received a standard regimen of 12.0 mg. of intramuscular betamethasone (Regimen B) concomitant with the ethanol infusion and an equal second dose 24 hours later. The other seven women received a 1,000 mg. intravenous dose of cortisol (Regimen C) initially and at 8, 16, and 24 hours thereafter.

Serial samples of maternal antecubital venous blood were obtained twice prior to corticoid administration and at 1, 4, 8, and 12 hours following the initial dose. The serum was stored at −20° C. Unconjugated E_3 levels were then determined by a specific radioimmunoassay.[3]

*The relative glucocorticoid potency of cortisol is 1/20 that of betamethasone.

The rate of corticoid delivery to the fetal hypothalamic-adrenal axis was estimated by the per cent suppression of maternal unconjugated estriol levels*[4] following drug administration. Least-squares regression lines ($P < 0.01$) were fitted to the log per cent suppression for each regimen. Regimen B was then compared to Regimen C. The time saved (Δt) by the use of Regimen C was obtained by calculating the difference in hours between Regimens B and C in attaining a comparable per cent suppression.

Results

Table I summarizes post-treatment suppression of unconjugated estriol mean per cent (±S.E.M.) pretreatment values. The difference between regimens obtained at 1 hour is highly significant at $P < 0.001$. At 4 hours, the betamethasone response is somewhat erratic and values obtained are not quite significant ($0.05 < P < 0.1$). The 8 and 12 hour values, however, are significant at $P \leq 0.5$.

Fig. 1 depicts the log per cent E_3 suppression vs. time and demonstrates the difference between regression lines for Regimen B as opposed to Regimen C. Regimen C produces an equivalent pharmacologic effect 9.0 ± 0.2 S.E.M. hours (Δt) more rapidly than does Regimen B.

*Unconjugated E_3 half-life is approximately 20 minutes.

Table II. Clinical data from those patients treated with betamethasone*

Patient	*Age*	*Gravida*	*Para.*	*Estimated gestational age (wk.)* †	*Comments*
M. O. M.	25	3	1	34	Delivered 28 hours after admission protocol. Weight 2,150 grams. Apgar 9/9. No IRDS
D. C.	20	1	0	31	Delivered 14 hours after admission protocol. Weight 850 grams. Apgar 1/5. Died 6 days of age secondary to complications of cardiac catheterization
E. M.	17	1	0	32½	Delivered 38 hours after admission protocol. Weight 2,220 grams. Apgar 3/7. No IRDS
P. K.	20	1	0	30½	Delivered 72 hours after admission protocol. Weight 1,001 grams. Apgar 2/7. No IRDS
D. L.	21	2	0	31	Delivered at 39 weeks' gestation. Weight 2,875 grams. Apgar 9/9. No IRDS
M. W.	21	7	1	33	Delivered at 40 weeks' gestation. Weight 3,200 grams. Apgar 7/9. No IRDS
L. W.	21	3	0	34	Delivered at 36 weeks' gestation. Weight 2,001 grams. Apgar 8/10. No IRDS

*No IRDS was encountered in any of these patients. One neonatal death occurred in this group but was unrelated to IRDS.
†Based on LMP and, where possible, measurement of fundal height, quickening, and first appearance of fetal heart tones.

Table III. Clinical data from patients treated with intravenous cortisol*

Patient	*Age*	*Gravida*	*Para.*	*Estimated gestational age (wk.)* †	*Comments*
D. C.	25	2	1	34	Delivered 32 hours after admission protocol. Weight 2,420 grams. Apgar 9/10. No IRDS
I. R.	25	2	1	33	Delivered 68 hours after admission protocol. Weight 2,380 grams. Apgar 8/8. No IRDS
C. H.	24	3	2	32	Delivered 23 hours after admission protocol. Weight 1,590 grams. Apgar 7/8. Mild IRDS
M. I. M.	23	3	1	28	Delivered 50 hours after admission protocol. Weight 960 grams. Apgar 2/5. Died 11 days after delivery of necrotizing enterocolitis. No IRDS
B. A	31	3	1	34	Delivered at 35 weeks. Weight 1,920 grams. Apgar 7/8. No IRDS
B. E. A.	23	1	0	33	Delivered 4 days after admission protocol. Weight 1,899 grams. Apgar 9/9. No IRDS
B. R.	24	4	3	30	Delivered at 33 weeks. Weight 2,013 grams. Apgar 2/7. Died 12 days after delivery of complications of closure patent ductus arteriosus. RDS present but related to cardiovascular complications

*Two neonatal deaths occurred in this group of cases, both unrelated to IRDS.
†Based on LMP and, where possible, measurement of fundal height, quickening, and first appearance of fetal heart tones.

Tables II and III summarize pertinent clinical data. Although the incidence of IRDS was negligible in both treatment series, one death occurred in the betamethasone group and two in the cortisol group—all from miscellaneous causes unrelated to IRDS.

Comment

It is reasonable to assume that glucocorticoid therapy for the prevention of IRDS in premature labor should be increasingly effective by use of a more rapidly acting regimen. These data indicate that Regimen C delivers a transplacental glucocorticoid effect comparable to Regimen B but with a saving in time of approximately 9 hours. In the untimely clinical setting of premature labor, such a difference could be crucial.

The therapeutic effectiveness of Regimen B, Regimen C, or glucocorticoids, in general, for accelerating fetal lung maturation is neither supported nor negated by this study. Several patients subsequently delivered at mature gestational age and were therefore not at risk

for IRDS. Moreover, the total number of pregnancies studied was small.

We continue to be in agreement with other investigators[6] that potential benefits of antenatal corticoid therapy significantly exceed the risks. Also, it is felt that further clinical investigation of the large-dose intravenous cortisol regimen is certainly warranted. It is our intent, however, that this drug regimen be used under conditions of strict supervision and then only on a protocol basis.

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Intrauterine Development and Fetal Management

Physicians are cordially invited to attend a 2½ day postgraduate course on "Intrauterine Development and Fetal Management" presented by the Johns Hopkins Medical Institutions on Thursday, Friday, and Saturday, April 22 through 24, 1976.

Members of the Johns Hopkins faculty and guests will discuss basic mechanisms of intrauterine development and physiology. This year, special emphasis will be given to newer developments in fetal monitoring, including fetal respiratory activity, the oxytocin challenge test, and pulse rate variability, and to immunologic relationships between the maternal and fetal units. The visiting faculty includes Drs. E. J. Quilligan, Leon I. Mann, Alan E. Beer, Charles W. Hohler, and Harold E. Fox.

This year's conference will be held at the beautiful Hunt Valley Inn, Baltimore County, Maryland. Registration will be limited to the first 125 applicants. For additional information, contact: Dr. John W. C. Johnson, Department of Gynecology and Obstetrics, The Johns Hopkins Hospital, Baltimore, Maryland 21205.

Placental aging monitored by gray scale echography

C. C. FISHER, M.R.C.O.G.
WILLIAM GARRETT, M.D.
GEORGE KOSSOFF, M.E.
Sydney, Australia

With gray scale ultrasonic echography, it is possible to identify changes in placental anatomy which formerly have been recognized only by examination of the placenta after delivery. By serial examinations these changes can be detected as they occur. The significance of the changes is discussed and an association between the premature appearance of aging of the placenta and a decline in placental function is noted.

THE GENERAL role of the placenta in the maintenance of pregnancy is well recognized but in recent years research has tended to concentrate on certain specialized aspects of its function. Anatomic examination of the placenta after the birth of the baby is an academic exercise from which retrospective correlations have been made between the condition of the infant and the gross and microscopic examination of the placenta. Ultrasound has now progressed to a stage where macroscopic information regarding the placenta, as well as the fetus, is available throughout pregnancy.[1]

Materials and methods

Five thousand consecutive series of gray scale echograms taken with Ultrasonics Institute abdominal echoscopes were examined and selected placentas were obtained following vaginal delivery, hysterotomy, or cesarean section. The placentas were washed, weighed, refrigerated, and within 24 hours were orientated into the position they occupied in the uterus.[2] They were then x-rayed and photographed before and after being cut into slices 1 cm. thick corresponding to the levels of the transverse ultrasonic tomograms. The x-ray examinations were made with a Siemens Pleophos four-mammogram unit with an Iontomat using 26 to 34 KV. at 800 Ma seconds with an exposure of 2 to 4 seconds.

From the Royal Hospital for Women and the Ultrasonic Institute.

Received for publication June 10, 1975.

Accepted July 10, 1975.

Reprint requests: Dr. C. C. Fisher, Ultrasonic Institute, 5 Hickson Road, Millers Point, 2000 N.S.W., Australia.

Results

Normal pregnancy. Early in pregnancy the tissue from which the placenta ultimately evolves, the chorionic villi, and decidua are readily recognizable as a white area lining the grayish myometrium and surrounding the spherical black echo-free space which corresponds to the amniotic sac and enclosed fluid. Fig. 1 shows a vertical and tranverse section from the same patient in which an 18 mm. (8 weeks of amenorrhea) embryo can be seen. This information is consistently obtained with gray scale echography.

An example of the normal development of placental ultrasonic appearance from this time on is shown in Fig. 2, which shows transverse section echograms through the placenta taken from the same patient at intervals throughout her pregnancy. After 10 weeks of amenorrhea the placental tissue takes on a uniform granular appearance. This appearance is associated with changes in the tissue density at a time when the placental lobules and the interlobular septa have not evolved to a stage where variations in acoustic impedance are apparent (Fig. 2, *a*).

As pregnancy progresses the amnion and chorion become apparent as a white line, indicating higher acoustic impedance, separating the placental tissue from the echo-free amniotic fluid. The membranes are seen initially between weeks 18 and 22 but may be identified earlier in some instances. At the same time the volume of the placenta increases as the uterus enlarges and the appearance of the placental tissue becomes more homogeneous in texture (Fig. 2, *b*) as the typical early granular appearance disappears. At this stage it is also possible to identify echo-free spaces behind the placenta. These spaces enlarge with time and it is thought that they represent sinuses in the choriodecidual space (Fig. 3, *a* and *b*).

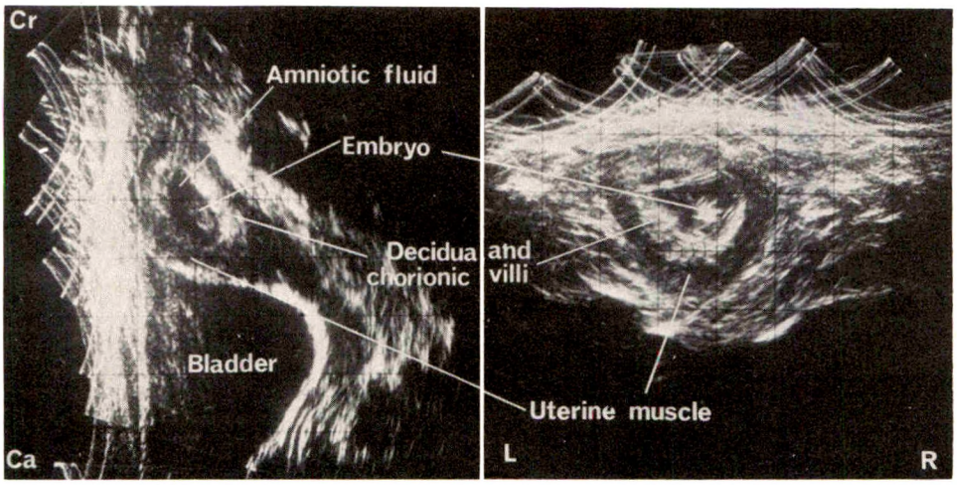


Fig. 1. Vertical and transverse echograms showing decidua, chorionic villi, and 18 mm. embryo.

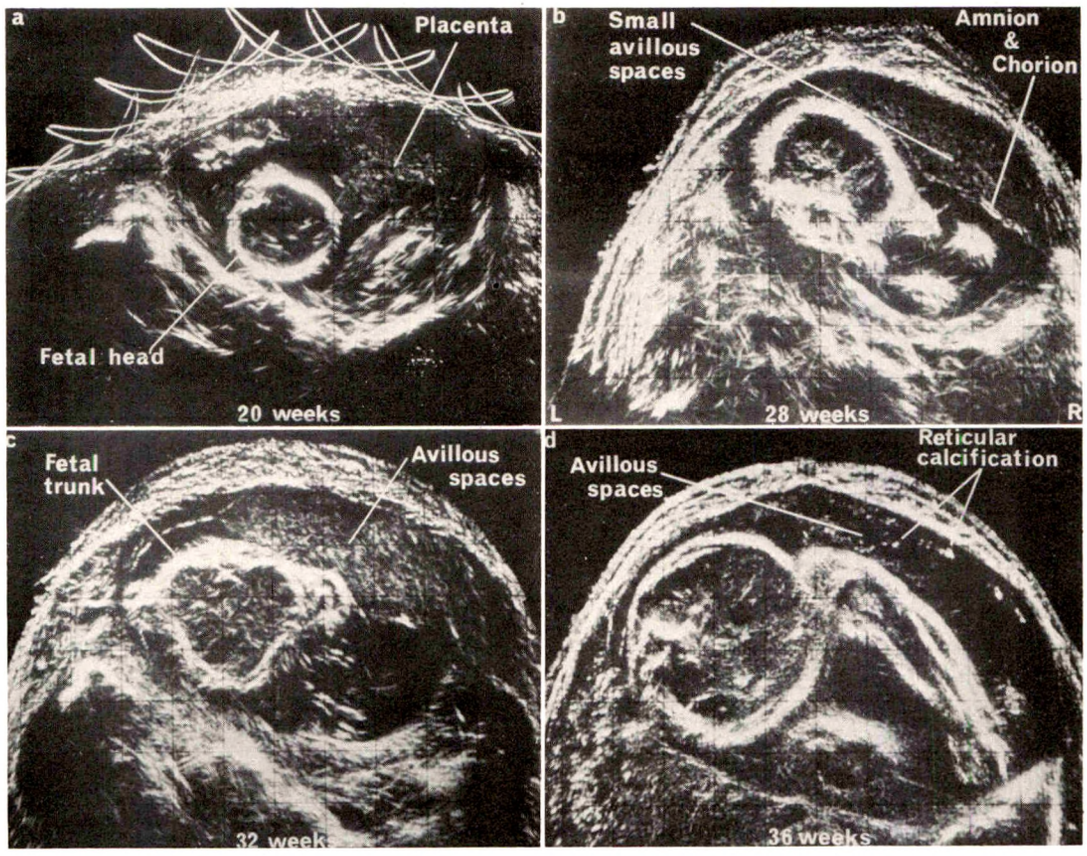


Fig. 2. Serial transverse scans from the same patient showing changing placental structure during a normal pregnancy.

At approximately 28 weeks of amenorrhea small echo-free spaces become apparent in the placental tissue (Fig. 2, *b* and *c*). These remain and enlarge as the pregnancy progresses toward term. After 36 weeks these spaces are readily detectable and become separated from each other by white areas corresponding to the intercotyledonary septa which become more marked in succeeding weeks (Fig. 2, *d*). The echo-free

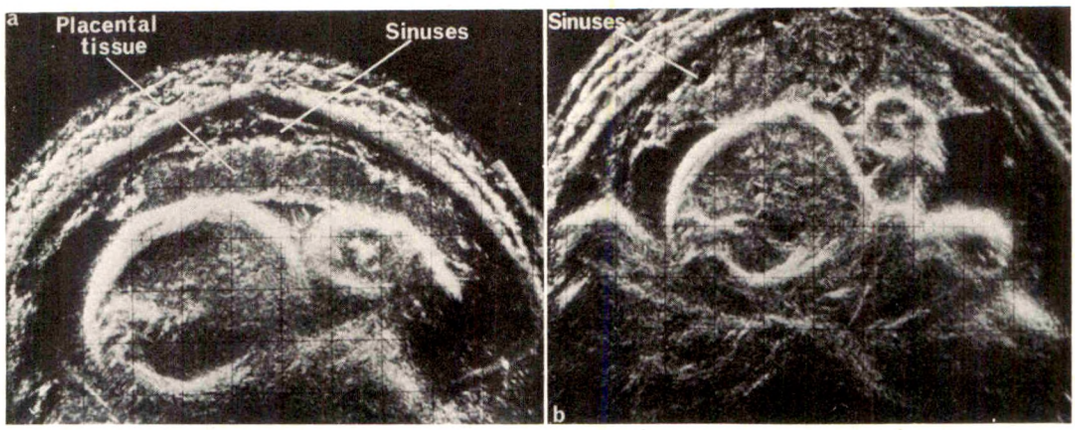


Fig. 3. Transverse echograms showing echo-free retroplacental spaces.

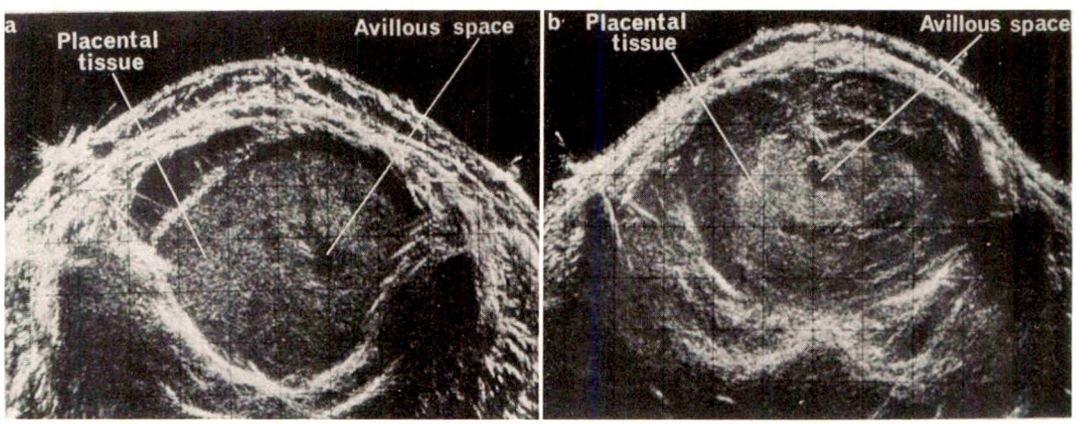


Fig. 4. Transverse echograms showing avillous spaces in placentas lying (*a*) in the lower uterine segment and (*b*) in the uterine fundus.

spaces are demonstrable in transverse section through placentas lying both vertically and horizontally within the uterus (Fig. 4, *a* and *b*).

It has been possible to correlate the ultrasonic findings with examination of the placentas after delivery. Echo-free spaces in the center of the lobules occurring from 28 weeks onward correspond to anatomic "holes" which in the fresh placenta are filled with blood (Figs. 5 and 6). Histologic examination shows that these spaces are virtually free from villi and that their size varies from 0.5 to 3 cm. in diameter. Their enlargement toward the end of pregnancy corresponds to a decline in the volume of amniotic fluid.

The strong echoes seen in the placentas during the last 3 to 4 weeks of pregnancy were found to correspond to the fibrous septa separating the lobules. X-ray and histologic examination shows that reticular calcification occurs at this stage within these septa and that it is from these small deposits of calcium that the strong echoes (white areas) arise (Figs. 5 and 6).

Another characteristic recorded occasionally is the lifting of the fetal membranes from the main mass of placental tissue on the fetal surface of the placenta. The spaces between the membranes and placenta are echo-free and these appearances are characteristic of choriol cysts (Fig. 7).

Abnormal pregnancy. In some pregnancies the ultrasonic characteristics here described occurring in late pregnancy have occurred at an earlier stage in pregnancy than one would normally expect. When this has been so the baby has been small for dates at delivery. An example is given in Fig. 8, which shows serial echograms from the same pregnancy. All of these sections represent echograms at the level of the fetal kidneys.

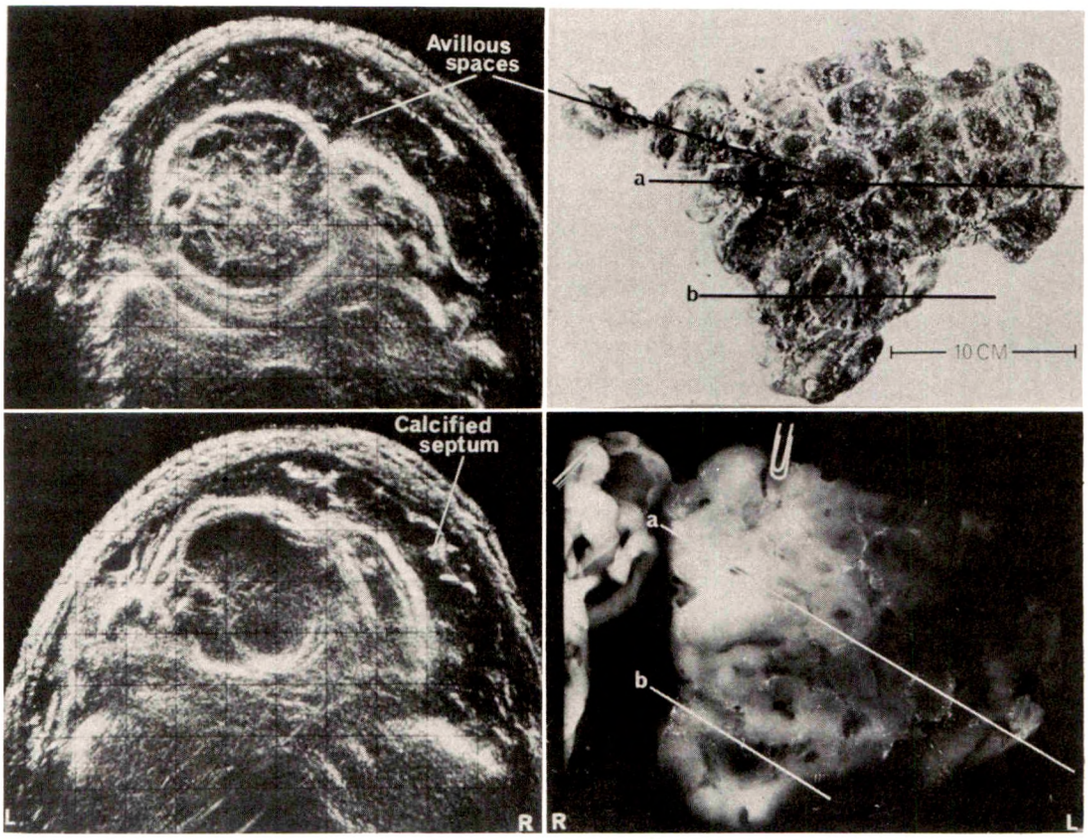


Fig. 5. Avillous spaces demonstrated by ultrasound, x-ray, and macroscopic inspection of the cut placenta.

In the echogram (Fig. 8, *a*) small echo-free spaces are visible in the placenta which is situated on the left of the anterior uterine wall. The spaces are mainly subchoriol and the remainder of the placental tissue shows the typical homogeneous appearance for this stage of pregnancy. The volume of amniotic fluid is still normal. With subsequent enlargement of the echo-free spaces and the appearance of increased amounts of calcification, the volume of amniotic fluid has diminished. Serial ultrasonic measurements of the fetal head and trunk show slowing of fetal growth and this was accompanied by falling estriol levels in 24 hour urinary assays. The baby showed clinical features of fetal growth retardation.

Comment

Reticular calcification is found in most placentas in increasing amounts toward term[3] and is more commonly seen in primigravidas than multigravidas. Crawford[4] has described holes in the center of the placental cotyledons and has found that they correspond to areas of entry of the maternal arterial jet under pressure. Histologically these areas are devoid of villi and accordingly we have used the term "avillous space" to describe them. The presumed role of these spaces is to increase the amount of blood flowing through the intervillous space to keep pace with the rapid growth of the fetus during late pregnancy.

Winsberg[5] has described a distinct ultrasonic appearance of the placenta occurring after 36 weeks' gestation which is marked by the appearance of "rounded transonic areas." These areas appear to correspond to the placental tissue and avillous spaces contained between the interlobular septa which show as white echoes due to their calcium content. He was not, however, able to correlate his findings by subsequent examination of the placentas which were "not considered unusual for term gestations." Detailed definition of placental structure can be made only with the use of gray scale echography and is not detectable with the nongray instruments that are more frequently used.

When reticular calcification and avillous spaces are found before the thirty-sixth week it may represent a need for greater placental blood flow in situations where the fetus is in jeopardy. Reduction in the volume

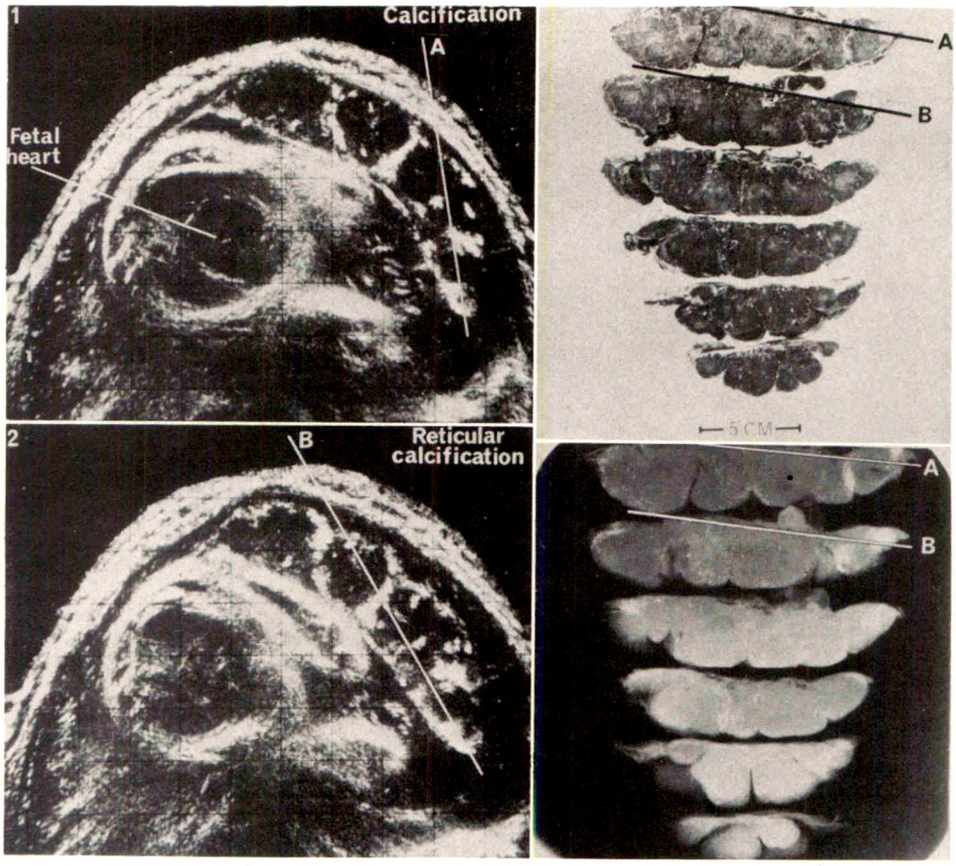


Fig. 6. Transverse echograms and placental x-rays from the same patient showing "strong" white echos corresponding to reticular calcification within the interlobular septa. Cuts *A* and *B* on the echograms correspond to those on the fixed specimen and x-ray.

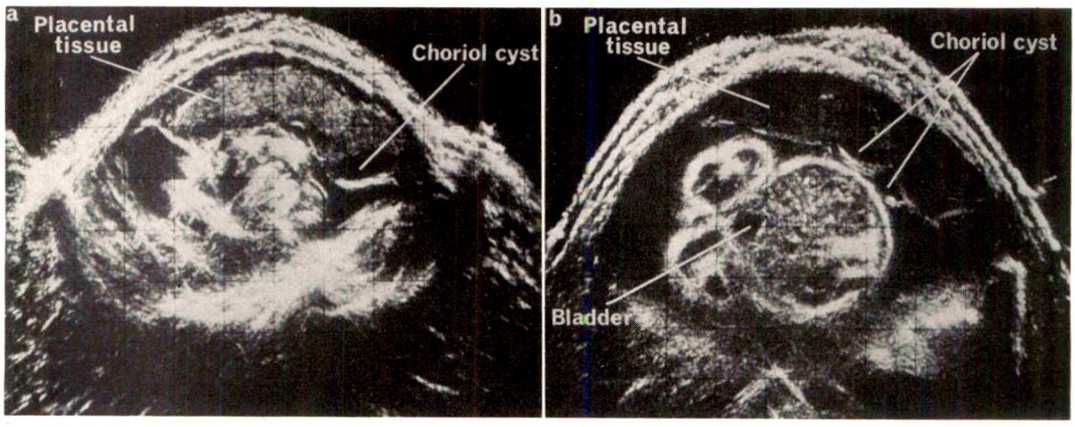


Fig. 7. Transverse echograms showing choriol cysts.

of liquor has been shown to occur normally in late pregnancy by means of dye dilution[6, 7] and this is shown ultrasonically; this reduction also occurs earlier in pregnancy when reticular calcification and avillous spaces appear prematurely.

Conclusion

It is shown that the ultrasonic placental appearances change throughout pregnancy and that these changes follow a fairly strict temporal sequence. This information can be useful when differentiating whether or not

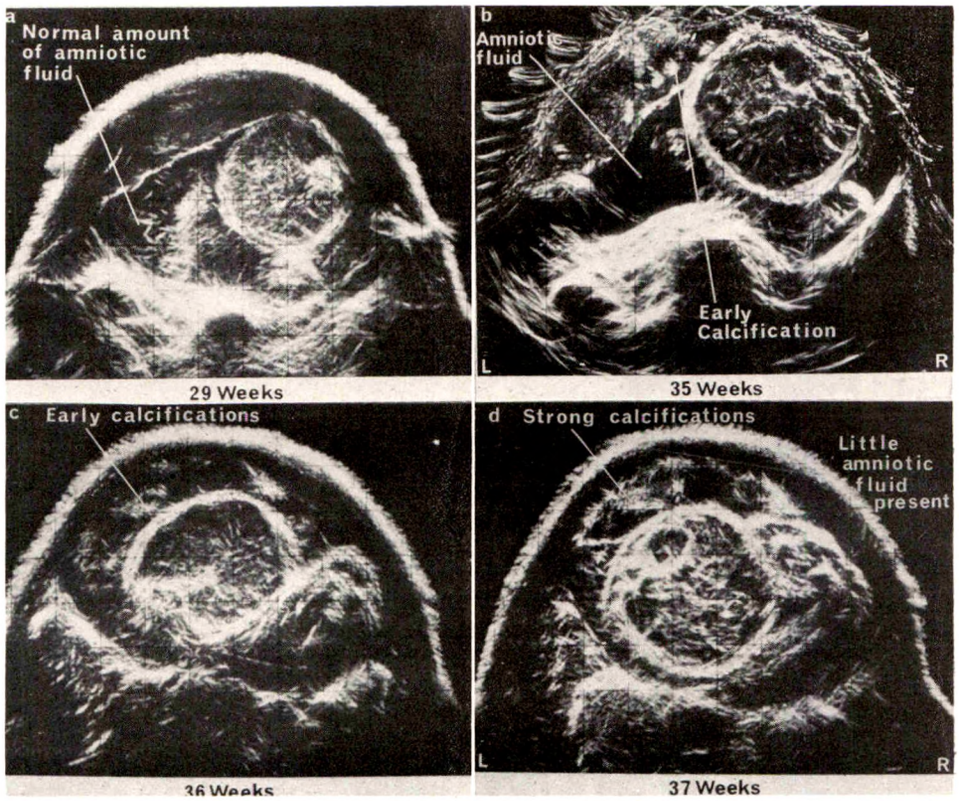


Fig. 8. Transverse serial echograms from the same patient showing early development of avillous spaces, reticular calcification, and reduction of amniotic fluid volume.

a fetus is small or large for its gestational age in instances when duration of pregnancy is not known or has been inaccurately assessed clinically. An example has been given where the change of placental appearance has been accelerated with an associated decline in placental function as evidenced by falling urinary estrogen levels and the ultimate answer, a growth retarded baby.

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Purification of naturally occurring antibody to human placental lactogen by affinity chromatography

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Antibodies against human placental lactogen (HPL) were isolated from maternal postpartum sera and nonpregnant female sera by the technique of immunoadsorption (or affinity chromatography). HPL was linked to Sepharose gel by cyanogen bromide activation and the resultant chromatographic resin was used in a repetitive column procedure to absorb and fractionate naturally occurring antibodies to HPL. The antibody to HPL was concentrated after elution from the column with a chaotropic ion, and tested for immunologic activity. It was determined to be an IgG molecule, and only radioimmunoelectrophoresis was sensitive enough, among the several tests performed, to show specific anti-HPL activity after treatment.

THE TECHNIQUE of affinity chromatography has proved to be an effective tool in the fractionation and purification of various proteins including antibodies.[1-3] This procedure utilizes the unique property that certain substances will specifically and reversibly bind to others. By irreversibly (covalently) binding a protein to a suitable gel such as Sepharose 6B* the resultant gel-protein complex may be used to separate specific antibodies directed against the bound protein from solutions such as sera, amniotic fluid, etc.

Naturally occurring antibody directed against HPL has previously been detected in the sera of some pregnant and postpartum patients by a hemagglutination assay.[4, 5] More recently, antibodies prepared against HPL have been isolated from the sera of immunized rabbits by affinity chromatography.[6] Our laboratory has utilized affinity chromatography in a modification of an automated column system for the purification of naturally occurring anti-HPL from human sera.[7] The relative effectiveness of this system and related procedures is reported.

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**Pharmacia, Inc., Piscataway, New Jersey.*

Materials and methods

Antigens and antisera. Purified HPL (717-340)* was used as an antigen in immunoassays and for immunizations of rabbits. Albino adult rabbits were each injected into the hind footpads and along the back with 1 ml. of solution containing 10 mg. of HPL mixed with 1 ml. of complete Freund's adjuvant (CFA). After 1 week the rabbits were injected again with the same amount of HPL mixed with an equal volume of incomplete Freund's adjuvant; this procedure was again repeated on the third week, and on the fourth week the rabbits were bled from the margin ear vein and serum was extracted, pooled, and stored at −20° C. The rabbit antibodies were used to check the standardization of different batches of HPL-sensitized formalinized red blood cells by hemagglutination, and to test the binding activity of the HPL immunoadsorbent column. Goat antihuman serum and antihuman IgG, IgA, and IgM were purchased.† These antisera were used at a concentration of 2 mg. per milliliter for the identification of the type of protein which was bound to the immunoadsorbent column. Immunodiffusion and im-

*Kindly supplied by Dr. P. H. Bell, Lederle Laboratories, Pearl River, New Jersey.

†Hyland Laboratories, Los Angeles, California.

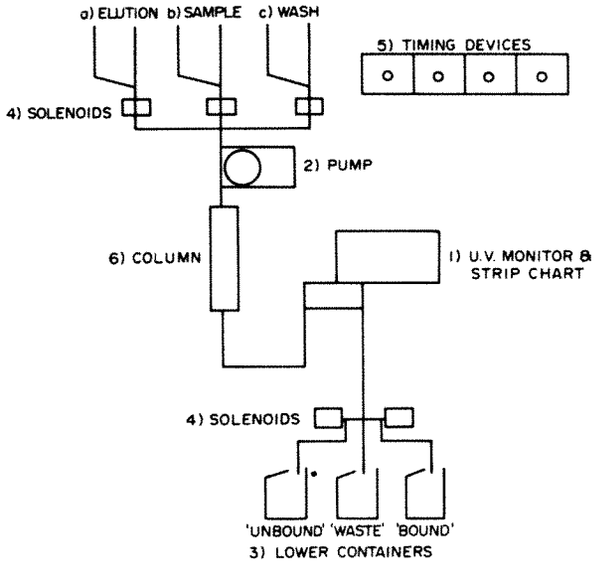


Fig. 1. Automated column system.

munoelectrophoresis was used as previously described.[8, 9]

Patient sampling. Blood samples were obtained from 38 patients. Twenty-six patients were 1 to 3 days post partum and 12 patients were nonpregnant women. Serum was extracted and either stored at −20° C. or assayed on the day of collection. The postpartum patients ranged in age from 15 to 32 years. Of the 12 nonpregnant patients studied, six had been pregnant at least once, and six denied pregnancy. The age range of these patients was from 18 to 32.

Hemagglutination. Sensitization of formalinized tanned sheep red blood cells (SRBC) with HPL was performed as previously described, at pH 6.4, with 0.25 per cent normal rabbit sera.[8] Sensitized SRBC's were tested with known rabbit antisera to HPL before assays with either patient's serum or protein bound to the HPL column, and then eluted and concentrated.

Immunoadsorption. The immunoadsorbent column was prepared by coupling 249 mg. of HPL to 45 ml. of wet Sepharose 6B. The gel was activated with 5 Gm. of Cyanogen bromide* for 10 to 15 minutes at pH 11 to 11.5 at a temperature of 18 to 25° C. before the addition of the ligand. The ligand and activated gel were slowly mixed by tumbling together overnight at 4° C. The pH had been adjusted to 9.0 before mixing. The gel was then washed with 1 L. of 0.1M (pH 7.2) Tris-HCl buffer and 1 L. of 0.1M (pH 9.0) sodium borate buffer. The optical density at 280 nm. of the wash buffers collected was found to be less than 0.002.

*Eastman Kodak, Rochester, New York

Coupling efficiency was determined by the formula:

$$1 - \frac{\text{O.D.U.'s of unbound HPL}}{\text{O.D.U.'s of total HPL}} \times 100\%$$

and was found to be greater than 95 per cent. The Sephadex gel-HPL was then degassed and gently poured into a 2.5 by 40 cm. glass column. This chromatographic column was then connected to the automated system.

Electrophoresis. Polyacrylamide gel electrophoresis (PAG) of proteins bound to the HPL-gel column was performed according to the method of Davies,[10] with 150 μg of each protein sample. Immunodiffusion was performed as previously described[9] with 1.5 per cent agar at a pH of 8.2. Immunoelectrophoresis was also performed as previously described[9] at pH 8.2 for 1 hour and 45 minutes at a constant 250V with the same agar as that which we used with immunodiffusion. All eluted proteins which had been bound to the HPL gel column were studied for anti-HPL activity. Immunoelectrophoresis with antihuman sera, as well as immunodiffusion with specific antisera against immunoglobulins G, A, and M were performed after the proteins had been dialyzed against pH 7.2 phosphate-buffered saline (PBS) and reconcentrated to the original volume of the sera applied to the column.

Automated column procedure. The automated column system (Fig. 1) consists of (1) UV monitor with strip chart recorder (ISCO),* (2) a peristaltic action variable flow adjustment pump (Buchler)† (3) upper and lower containers (4) solenoid switches, (5) timing devices, and (6) a chromatographic column.

The timing devices control the solenoid switches which, in turn, determine which solution will flow onto the column and into which vessel the effluent will be collected. The UV monitor records the optical density, at 280 nm., of the solution leaving the column, while the pump maintains a steady flow rate. The upper vessels are filled with (1) 1.5 M SCN elution buffer, (2) sample, and (3) 0.01M Tris-HC wash buffer. The lower vessels are used to collect waste and solutions "unbound" and "bound" to the column.

A 5 ml. quantity of serum is placed in the sample container and allowed to flow onto the column. The timing devices control the upper three solenoids and bottom two sliding solenoids simultaneously. The timing devices are set to allow "wash" buffer to wash the serum through the column. All "unbound" material passes through the column and is collected in the "unbound" container. After the column is washed clean of "unbound" material as indicated by a zero UV

*Instrumentation Specialties Co., Lincoln, Nebraska.
†Buchler Instruments, Fort Lee, New Jersey.



Fig. 2. The result of radioimmunoelectrophoresis utilizing iodinated HPL in the upper well and nonradioactive HPL in the lower well. The trough was filled with IgG from a postpartum patient which had been bound to the affinity chromatography column, dialyzed, and concentrated. Some residual antibody activity is still seen.

monitor reading, the next timer causes the elution buffer to flow onto the column by switching the opening to that container, and closing the former. The chaotropic elution buffer disrupts the antigen-antibody bonds which cause the protein to bind to the HPL-gel and this solution is then washed into the bound collection vessel. Finally, when no more protein is being released from the gel the next timer opens the last solenoid which controls the wash buffer and the column is washed free of the "elution" buffer and this is collected in the "waste" container. The bound material was concentrated by ultrafiltration on an XM-100 membrane* and dialyzed against wash buffer before testing it for the presence of antibody activity to HPL. It is necessary to run the first two samples by hand, in order to determine how long each phase takes. Then the times could be set on the timers and all further runs were automatic. This automatic technique is useful only when the same sample solution is used; then the column is run automatically. For our purposes, in this study, however, each sample serum size was the same, and therefore the timing was the same. Each individual sample was applied by hand. Each cycle required 1.5 hours.

Radioimmunoelectrophoresis. Radioimmunoelectrophoresis was performed as described previously.[9] HPL was iodinated[11] with I^{125} and immunoelectrophoresis was performed against "bound" protein. HPL-I^{125} was placed in the outside wells and electrophoresed for 1 hour and 45 minutes at 250 volts. "Bound" protein (in some instances this was pooled) was placed in the center troughs and allowed to immunodiffuse for 24 hours at 37° C. The slides were successively dialyzed in saline and distilled H_2O and then dried. The dried agar slides were incubated in the dark against type N-S Kodak x-ray film for 4 days and developed by standard procedures.

*Amicon Corporation, Lexington, Massachusetts

Results

Pooled serum from rabbits immunized against HPL was tested by hemagglutination for anti-HPL and was found to have a titer of 1/1,280. The rabbit serum was then used to test the immunoadsorbent column. Samples were placed on the column and "bound" solutions when tested demonstrated titers of 1:320 when the 1.5M SCN was used as elution buffer, and the bound fraction had been reconstituted to the original sample size. Other previously tested elution buffers such as low pH acids or guanidine-HCl caused an even greater loss of immunologic activity.

The patient's sera were then tested by hemagglutination, immunodiffusion, and immunoelectrophoresis for anti-HPL activity before application to the immunoadsorbent column. Seven of these sera were found to be positive by hemagglutination and had titers of 1:2 to 1:16. These seven sera were from postpartum patients. All 38 sera were then chromatographed on the HPL-gel column and 37 demonstrated binding to the HPL-gel as indicated by an increased optical density in the elution buffer flowing from the column. The "bound" solutions were concentrated to their original serum volume, dialyzed against wash buffer, then tested for antibody activity against HPL by hemagglutination, immunodiffusion, and immunoelectrophoresis. All were found to be negative. Polyacrylamide gel electrophoresis of the samples showed homogeneity as indicated by single identical bands. The samples were then tested for human IgG, IgM, and IgA activity and all bound samples were identified as IgG.

All 26 postpartum patients demonstrated the presence of antibody activity by binding. Only one

patient's serum globulin did not bind. She was a normal 18-year-old patient with a history of never having been pregnant, and was being seen for a routine follow-up in the gynecology outpatient clinic.

The sensitive technique of radioimmunoelectrophoresis was then used. Single sample of "bound" solutions did not react against the HPL-I^{125}, but pooled samples gave precipitation lines. These results indicate the presence of antibody activity to HPL. Fig. 2 demonstrates a positive study.

Comment

Immunoadsorption with an automated column system has been successfully used to demonstrate the presence of naturally occurring antibodies against HPL in the sera of postpartum women. A significant loss of immunologic activity was observed in rabbit antisera and in postpartum sera as demonstrated by decreased hemagglutination titers after passage through the column. This loss of activity may be attributed to the relatively harsh treatment of the antibody by the elution buffer, sodium thiocyanate (SCN). Various elution buffer systems were tested, including guanidine-HCl and low pH acid buffers, before deciding upon SCN, which we found produced the least destructive results. Before column application of the sera, hemagglutination tests indicated the presence of antibody in seven of the patient's sera, while immunodiffusion and immunoelectrophoresis were negative. The presence of immunologic antibody activity against HPL was detected in the bound samples after passage through the column only by the very sensitive technique of radioimmunoelectrophoresis. Although we cannot be exact about the amount of protein actually detected in this system, the lower limit of detectability of the formation of immunoglobulins by radioimmunoelectrophoresis has been found to be 0.002 μg.[12]

Polyacrylamide gel electrophoresis of the bound material showed homogeneity of the bound samples, and immunoelectrophoresis and immunodiffusion demonstrated the presence of IgG only. These results indicate a successful separation of a single substance from the many proteins present in the human sera.

The lack of a binding protein in the serum of one postpartum patient is a curious phenomenon for which we have no explanation. The binding of proteins from the sera of nonpregnant females would indicate the presence of anti-HPL, probably the result of some previous contact with this placental antigen. There was no relationship between the presence of the antibody and parity or duration of gestation, or abortion. None of the patients tested had ever had trophoblastic disease.

Quantitative studies were not performed in this series of experiments but could be adapted easily to this system, in order to determine the relative amounts of anti-HPL. The levels of anti-HPL may be of some clinical significance in determining the status of the fetus or fetal-maternal immune response. We have not yet made sufficient progress to determine exactly what the significance of this phenomenon might be.

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A simple geometric method for determining fetal weight in utero with the compound gray scale ultrasonic scan

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A method to estimate intrauterine fetal weight by calculating fetal volume from simple parameters measured on echograms is described. The comparison between assessed weight of 50 fetuses and actual birth weight is presented.

PREVIOUS reports of estimations of fetal weight in utero by ultrasonic methods have proved to be inaccurate.[1, 2] Methods are now being developed requiring complex mathematical assessment and computer analysis. A simple calculation for fetal weight based on geometric principles in combination with ultrasonic measurement has been devised. The preliminary results have been of clinical significance.

The method involves localization in relation to the maternal midline of two parameters: the midpoint of the fetal skull, as determined by the falx cerebri, and the midpoint of the fetal bladder. Both points are recognized in transverse scans of the maternal abdomen. Longitudinal scans are then performed along the line joining the farthest extent of the fetal head to the pelvic floor as represented by the most distant extent of the fetal bladder. The distance between these two points is then measured in centimeters. The fetus is

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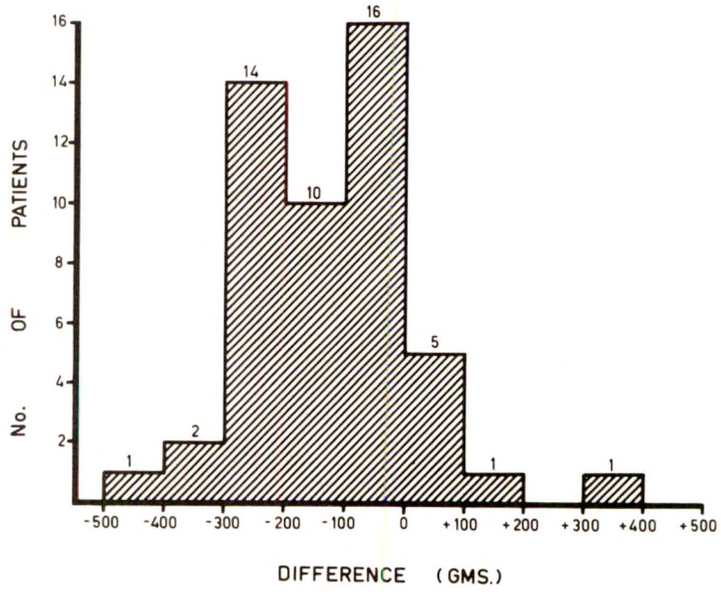


Fig. 1. Histogram of 50 patients grouped according to the difference between predicted and actual fetal weight. Difference recorded in 100 Gm. increments.

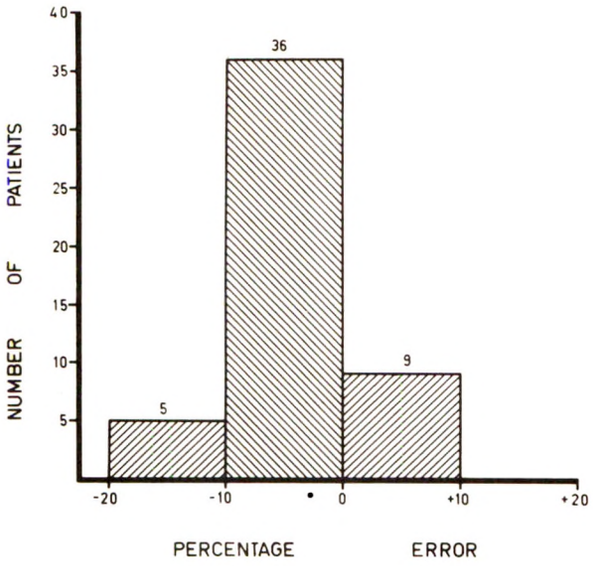


Fig. 2. Histogram showing percentage error between actual and predicted fetal weight. Difference recorded in 10 per cent increments.

then regarded as a cylinder whose volume is represented by the simple formula

$$\pi R^2 H,$$

where R = ½ biparietal diameter and H = the distance from tip of fetal head to inferior extent of fetal bladder.

The calculated volume of trunk and head is then multiplied by the specific gravity of the human body—that is, 1.067. The result is then obtained in grams.

The width of the fetal thigh at the upper end of the femur is then recognized and measured from horizontal scans of the fetal trunk. The length of the fetal thigh is then measured from an appropriate scan depending on the attitude of the limb. The fetal leg is then regarded as a cylinder whose volume is calculated by the same formula, where: R = ½ the width of the upper thigh and H = 2 × length from hip to knee.

This result is then multiplied by 4 to represent all four fetal limbs and again multiplied by the specific gravity of the human body. The result is then added to the trunk weight.

Measurement of neonatal limbs have demonstrated that the arm and leg is of approximately equal length, supporting the assumption that measurement of 1 leg can represent all fetal limbs.

The results found in 50 patients examined the day prior to induction of labor or cesarean section are shown in Fig. 1.

It can be seen from this that 90 per cent of the estimations lie within 400 Gm. of the actual birth weight, in fact 64 per cent lie within 200 Gm. of the actual birth weight.

Fig. 2 shows that 36 lie within 10 per cent of the actual birth weight and that 41 lie below the actual birth weight. The method tends therefore to slightly underestimate fetal weight, which is a safety factor in the management of small-for-dates infants. Furthermore, it has been observed that the method tends to be more accurate at the lower fetal weights, possibly because of the significant weight of the gluteal region of the larger fetus.

This method appears to be a valuable adjunct in the assessment of the premature or small-for-dates fetus. The study is being continued in order to refine the method and use it as an additional index of fetal growth.

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- two ratios to choose from—either 2.5 or 1 mg progestin—both effective and both combined with 50 mcg of estrogen
- a low incidence of annoying side effects which some patients find unacceptable with other dosages or products*
- a regimen with few problems—28-day non-stop—particularly well-suited for busy patients who have difficulty keeping track of the days

this year or any year

Norlestrin® Fe 2.5 mg

Each pink tablet contains 2.5 mg norethindrone acetate, 50 mcg ethinyl estradiol. Each brown tablet contains 75 mg ferrous fumarate, USP.

Norlestrin Fe 1 mg

Each yellow tablet contains 1 mg norethindrone acetate, 50 mcg ethinyl estradiol. Each brown tablet contains 75 mg ferrous fumarate, USP.

*Both serious and minor adverse reactions have been reported by women taking oral contraceptives. The physician should be alert to their manifestations and should carefully review the following page for prescribing information.

PARKE-DAVIS

See next page for brief summary of prescribing information.

Norlestrin
(norethindrone acetate and ethinyl estradiol tablets, NF)



BRIEF SUMMARY OF PRESCRIBING INFORMATION

ACTIONS

Norlestrin achieves its contraceptive effect primarily by inhibition of ovulation through gonadotropin suppression. It is possible that other sites of action such as changes in cervical mucus and in the endometrium may contribute to the high efficacy of oral contraceptives.

SPECIAL NOTE

Oral contraceptives have been marketed in the United States since 1960. Reported pregnancy rates vary from product to product. The effectiveness of the sequential products appears to be somewhat lower than that of the combination products. Both types provide almost completely effective contraception.

An increased risk of thromboembolic disease associated with the use of hormonal contraceptives has now been shown in studies conducted in both Great Britain and the United States. Other risks, such as those of elevated blood pressure, liver disease, and reduced tolerance to carbohydrates, have not been quantitated with precision.

Long-term administration of both natural and synthetic estrogens in subprimate animal species in multiples of the human dose increases the frequency for some animal carcinomas. These data cannot be transposed directly to man. The possible carcinogenicity due to the estrogens can neither be affirmed nor refuted at this time. Close clinical surveillance of all women taking oral contraceptives must be continued.

INDICATION

Norlestrin is indicated for the control of conception.

CONTRAINDICATIONS

1. Thrombophlebitis, thromboembolic disorders, cerebral apoplexy, or a past history of these conditions.
2. Markedly impaired liver function.
3. Known or suspected carcinoma of the breast.
4. Known or suspected estrogen-dependent neoplasia.
5. Undiagnosed abnormal genital bleeding.
6. Pregnancy.

WARNINGS

1. The physician should be alert to the earliest manifestations of thrombotic disorders (thrombophlebitis, cerebrovascular disorders, pulmonary embolism, and retinal thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately.

Retrospective studies of morbidity and mortality in Great Britain and studies of morbidity in the United States have shown a statistically significant association between thrombophlebitis, pulmonary embolism, and cerebral thrombosis and embolism and the use of oral contraceptives. There have been three principal studies in Britain[1-3] leading to this conclusion, and one[4] in this country. The estimate of the relative risk of thromboembolism in the study by Vessey and Doll[3] was about sevenfold, while Sartwell and associates in the United States found a relative risk of 4.4, meaning that the users are several times as likely to undergo thromboembolic disease without evident cause as nonusers. The American study also indicated that the risk did not persist after discontinuation of administration, and that it was not enhanced by long continued administration. The American study was not designed to evaluate a difference between products. However, the study suggested that there might be an increased risk of thromboembolic disease in users of sequential products. This risk cannot be quantitated, and further studies to confirm this finding are desirable.

2. Discontinue medication pending examination if there is sudden partial or complete loss of vision, or if there is a sudden onset of proptosis, diplopia, or migraine. If examination reveals papilledema or retinal vascular lesions, medication should be withdrawn.
3. Since the safety of Norlestrin (norethindrone acetate and ethinyl estradiol) in pregnancy has not been demonstrated, it is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period.
4. A small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The long-range effect to the nursing infant cannot be determined at this time.

PRECAUTIONS

1. The pretreatment and periodic physical examinations should include special reference to breasts and pelvic organs, including Papanicolaou smear, since estrogens have been known to produce tumors, some of them malignant, in five species of subprimate animals.
2. Endocrine and possibly liver function tests may be affected by treatment with Norlestrin. Therefore, if such tests are abnormal in a patient taking Norlestrin, it is recommended that they be repeated after the drug has been withdrawn for two months.
3. Under the influence of estrogen-progestogen preparations, preexisting uterine fibromyomata may increase in size.
4. Because these agents may cause some degree of fluid retention, conditions which might be influenced by this factor, such as epilepsy, migraine, asthma, cardiac or renal dysfunction, require careful observation.
5. In breakthrough bleeding, and in all cases of irregular bleeding per vaginam, nonfunctional causes should be borne in mind. In undiagnosed bleeding per vaginam, adequate diagnostic measures are indicated.
6. Patients with a history of psychic depression should be carefully observed and the drug discontinued if the depression recurs to a serious degree.
7. Any possible influence of prolonged Norlestrin therapy on pituitary, ovarian, adrenal, hepatic, or uterine function awaits further study.
8. A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. The mechanism of this decrease is obscure. For this reason, diabetic patients should be carefully observed while receiving Norlestrin therapy.
9. The age of the patient constitutes no absolute limiting factor, although treatment with Norlestrin may mask the onset of the climacteric.
10. The pathologist should be advised of Norlestrin therapy when relevant specimens are submitted.
11. Susceptible women may experience an increase in blood pressure following administration of contraceptive steroids.

ADVERSE REACTIONS OBSERVED IN PATIENTS RECEIVING ORAL CONTRACEPTIVES

A statistically significant association has been demonstrated between use of oral contraceptives and the following serious adverse reactions:

Thrombophlebitis
Cerebral thrombosis
Pulmonary embolism

Although available evidence is suggestive of an association, such a relationship has been neither confirmed nor refuted for the following serious adverse reactions:

Neuro-ocular lesions, eg, retinal thrombosis and optic neuritis

The following adverse reactions are known to occur in patients receiving oral contraceptives:

Nausea
Vomiting
Gastrointestinal symptoms (such as abdominal cramps and bloating)
Breakthrough bleeding
Spotting
Change in menstrual flow
Amenorrhea during and after treatment
Edema
Chloasma or melasma
Breast changes: tenderness, enlargement, and secretion
Change in weight (increase or decrease)
Changes in cervical erosion and cervical secretions
Suppression of lactation when given immediately postpartum
Cholestatic jaundice
Migraine
Rash (allergic)
Rise in blood pressure in susceptible individuals
Mental depression

Although the following adverse reactions have been reported in users of oral contraceptives, an association has been neither confirmed nor refuted:

Anovulation post-treatment
Premenstrual-like syndrome
Changes in libido
Changes in appetite
Cystitis-like syndrome
Headache
Nervousness
Dizziness
Fatigue
Backache
Hirsutism
Loss of scalp hair
Erythema multiforme
Erythema nodosum
Hemorrhagic eruption
Itching

The following laboratory results may be altered by the use of oral contraceptives:

Hepatic function: increased sulfobromophthalein retention and other tests; Coagulation tests: increase in prothrombin Factors VII, VIII, IX, and X; Thyroid function: increase in PBI, and butanol-extractable protein-bound iodine and decrease in T^3 uptake values; Metyrapone test; Pregnanediol determination.

SPECIAL NOTE

In the rare case of bleeding which resembles menstruation, the patient should be advised to discontinue medication and then begin taking tablets from a new compact on the fifth day (Day 5). Persistent bleeding which is not controlled by this method indicates the need for re-examination of the patient, at which time nonfunctional causes should be borne in mind.

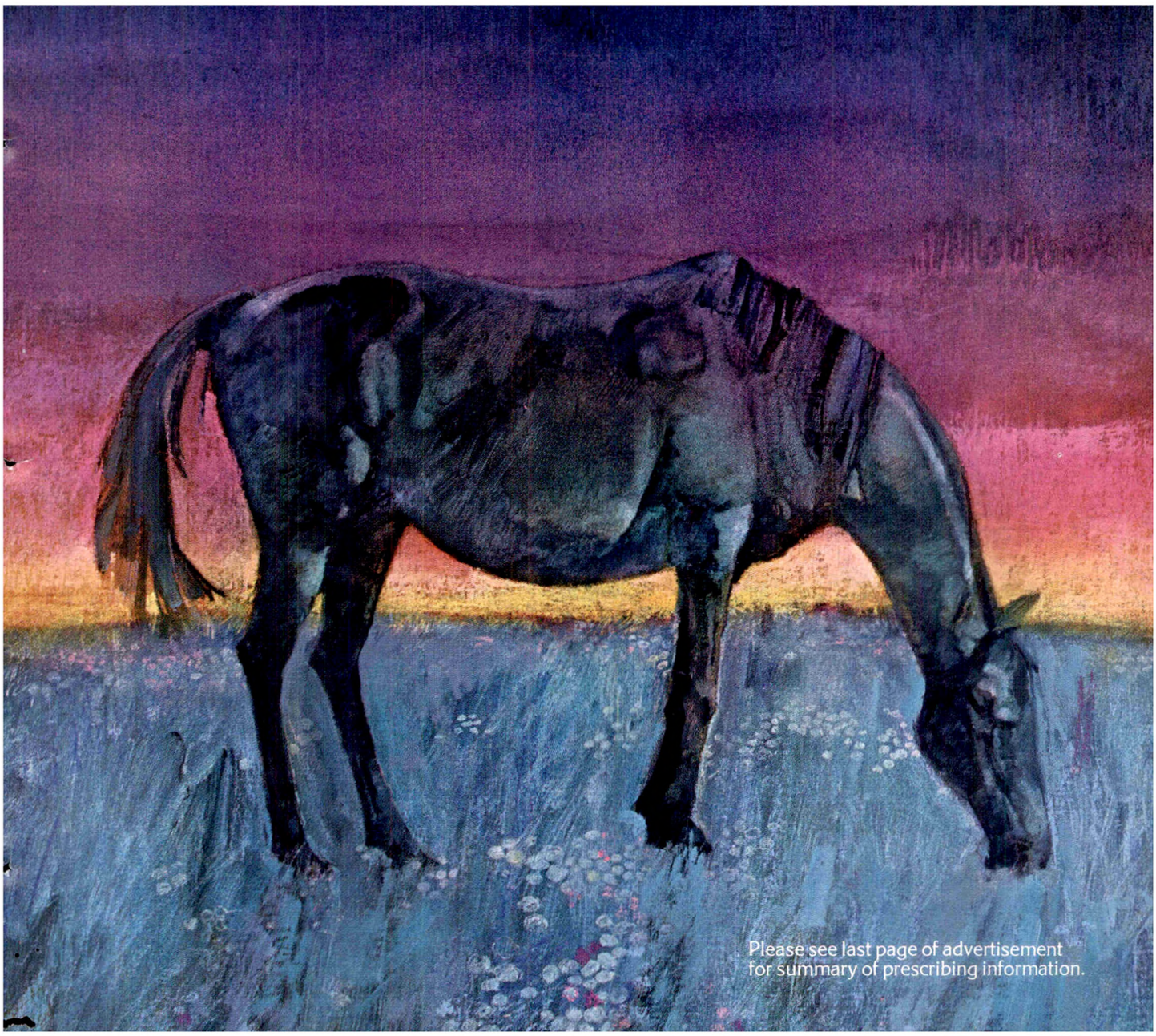
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Please see last page of advertisement
for summary of prescribing information.



The dawn of a new age

Genisis—The Reason for Change

Discovery, isolation, identification, synthesis—the classical pattern for creating drugs which once were available from natural sources only. Antibiotics, vitamins, and sex hormones come to mind as examples of drugs which have been developed and perfected in this manner. Obvious advantages of this evolution include: economy, standardization, reproducibility, and purity.

Natural Estrogens—A Mystique

There are those who would have us believe that conjugated estrogens, U.S.P. derived from the urine of pregnant mares is endowed with a certain mystique. What, precisely, is conjugated estrogens "natural"?

A study of the excretion pattern of pregnant-mare estrogens reveals that estrone and equilin are the major components and 17 alpha-dihydroequilin a minor one. Estrone predominates in early pregnancy but as this progresses equilin levels increase and may equal or surpass those of estrone in late pregnancy. This is believed to reflect continuing changes in the placental metabolism of estrogen.[1]

The urine of pregnant mares is pooled to compensate for these variations and provide a raw material of more or less average composition. This practice, however, has the disadvantage of providing a urine pool which contains all of the excreted estrogenic and related hormones whether active or not. For example, an assay study of the estrogenic content of a "large mixture" of mares' urine reveals the following proportions: 51% sodium estrone sulfate, 22% sodium equilin sulfate, 16% sodium 17 alpha-dihydroequilin, and smaller amounts of the sodium sulfates of equilenin, 17 alpha-dihydroequilenin and 17 alpha-estradiol. Related "non-estrogenic" steroids were also present.[2]

Despite these variations, conjugated estrogens, U.S.P. is defined as containing 50 to 65% sodium estrone sulfate and 20 to 35% sodium equilin sulfate.[3]

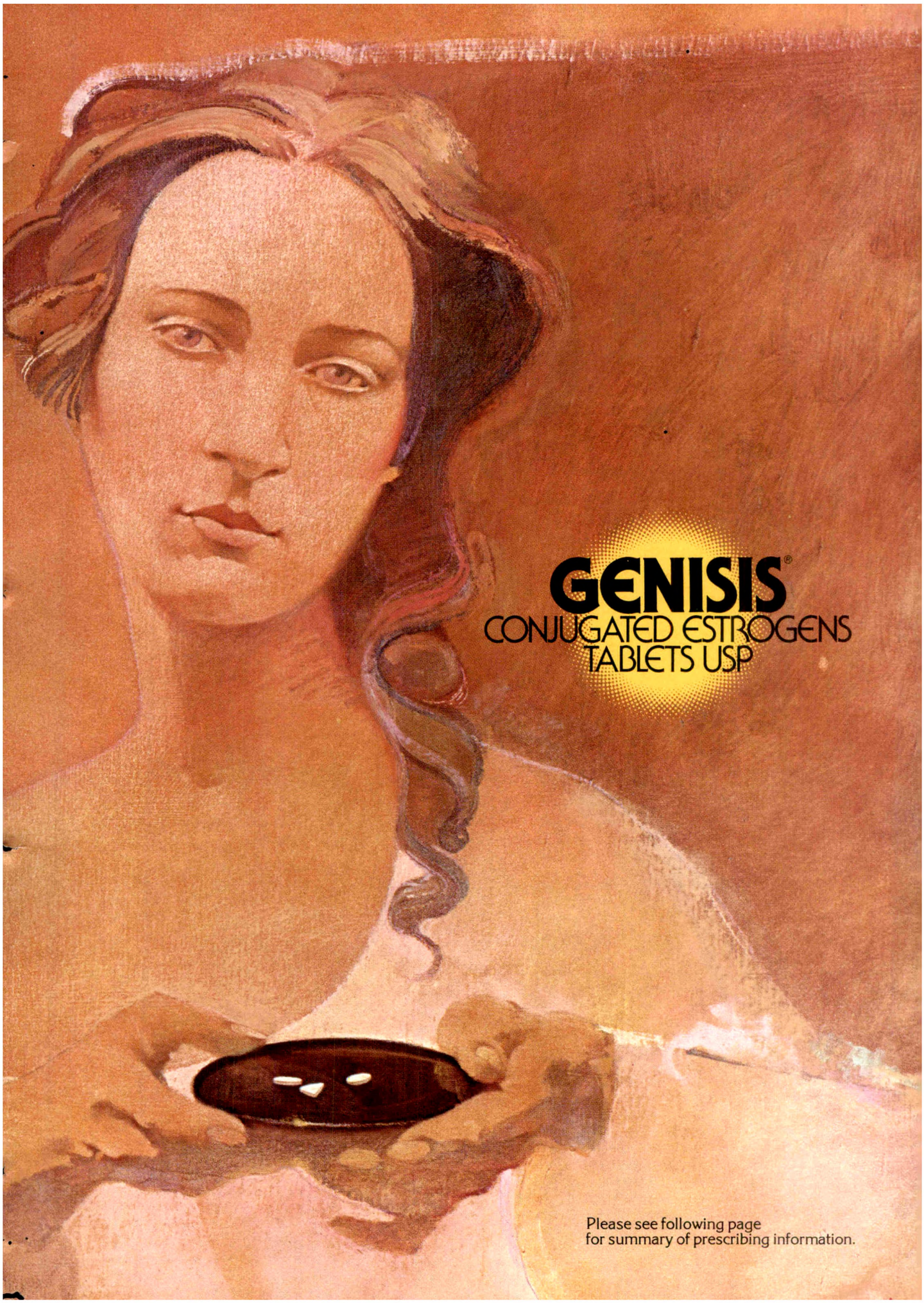
Genisis—A Beginning

With Genisis, the beginning of a new age of conjugated estrogens, U.S.P. preparations is here. It is free of extraneous natural residues such as "non-estrogenic" steroids. It is prepared synthetically and specifically formulated so that each tablet contains the following approximate proportions of active substances:

Sodium estrone sulfate—60%
Sodium equilin sulfate—30%
17 alpha-dihydroequilin sodium sulfate—10%

Genisis is indicated for those standard indications requiring conjugated estrogens.

Genisis is supplied in snow-white pure uncoated tablets in the usual potencies of: 0.625 mg/1.25 mg/2.50 mg.



Please see following page
for summary of prescribing information.

GENISIS®
CONJUGATED ESTROGENS TABLETS USP

BRIEF SUMMARY
Please consult full package circular before prescribing

Indications
Genisis is indicated for replacement therapy of estrogen deficiency associated with menopausal syndrome, female hypogonadism (hypogenitalism), amenorrhea, female castration or primary ovarian failure.

Important Notes on the Use of Estrogens
In view of the positive relationship between thromboembolic disease and the use of oral contraceptives containing estrogens and progestins, it would seem prudent and in keeping with basic therapeutic principles to utilize, whenever feasible, the smallest effective dose of estrogens in treating patients.

The possible carcinogenicity of estrogens cannot be confirmed or refuted at this time. Close clinical surveillance of all women taking estrogens must be continued.

Contraindications
Patients with, or with history, of:
markedly impaired liver function;
known or suspected carcinoma of breast (except progressing disease not amenable to surgery or irradiation in women at least 5 years postmenopausal, or in men in whom castration is not possible);
known or suspected estrogen-dependent neoplasia, such as carcinoma of the endometrium;
undiagnosed genital bleeding;
thrombophlebitis, thromboembolic disorders, cerebral apoplexy.

Warnings
Discontinue medication at the earliest sign of thrombotic disorders (thrombophlebitis, cerebrovascular disorders, pulmonary embolism, retinal thrombosis), or in the presence of papilledema, retinal vascular lesions or such untoward effects as progression of cancer or hypercalcemia. Discontinue pending examination if there is sudden onset of proptosis, diplopia or migraine.

A statistically significant association has been reported between maternal ingestion of diethylstilbestrol during pregnancy and the occurrence of vaginal carcinoma years later in the offspring. Whether such an association is applicable to all estrogens is not known at this time. In any event, estrogens are not indicated for use during pregnancy.

Estrogens excreted in mother's milk may affect the nursing infant.

Precautions
Certain patients may be unusually responsive and may respond with undesirable manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding, mastodynia, edema, etc. Because of possible salt and water retention, estrogens should be used with caution in patients with epilepsy, migraine, asthma, cardiac or renal disease. Estrogens influence the metabolism of calcium and phosphorus. They should be used with caution in patients with certain metabolic bone diseases that are associated with hypercalcemia or in patients with renal insufficiency. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Diabetic patients should be followed closely because of possible decrease in glucose tolerance.

In the event of unexplained or excessive vaginal bleeding during estrogen therapy, nonfunctional causes should be borne in mind. The drug should be discontinued and a thorough investigation made as to the cause, being certain to rule out the possibility of malignancy. Pre-existing uterine fibromyomata may increase in size when estrogens are used. Therefore, patients should be examined at regular intervals throughout therapy. Women with a strong family history of cancer, recurrent chronic mastitis, or abnormal mammograms should be administered estrogens with caution. A pre-treatment physical examination should include special reference to breast and pelvic organs as well as a Papanicolaou smear since estrogens have been known to produce tumors, some of them malignant, in five species of animals.

Continuous use of estrogens will result in prolonged stimulation of the endometrium and breast. In order to avoid this, oral estrogens should be administered cyclically in the menopausal or hypogonadal patient.

The pathologist should be advised of estrogen therapy when relevant specimens are submitted. Certain endocrine and liver function tests may be affected by treatment with estrogens. If such tests are abnormal in a patient taking these drugs it is recommended that they be repeated after the drug has been withdrawn for two months. Any possible influence of prolonged estrogen therapy on pituitary, ovarian, adrenal, hepatic or uterine function awaits further study.

When large doses of estrogens are used, urinary stress incontinence may occur in nonpregnant females.

Adverse Reactions
A statistically significant association has been demonstrated between use of estrogen-progestin oral contraceptives and the following serious reactions: thrombophlebitis, cerebral thrombosis, pulmonary embolism.

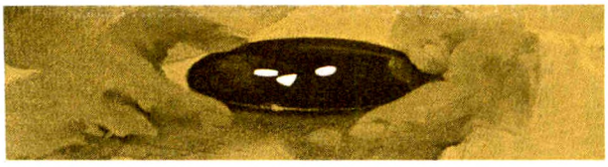
Although available evidence is suggestive of an association, such a relationship has been neither confirmed nor refuted for the following serious reactions: coronary thrombosis, neuro-ocular lesions (e.g. retinal thrombosis and optic neuritis).

The following adverse reactions are known to occur in patients receiving estrogens: nausea, vomiting, anorexia, gastrointestinal symptoms (such as abdominal cramps or bloating), edema, breakthrough bleeding, spotting or withdrawal bleeding, breast tenderness and enlargement, change in body weight (increase or decrease), headache, increased cervical mucus, allergic rash, loss of libido and gynecomastia in the male, reactivation of endometriosis, aggravation of migraine headaches, hepatic cutaneous porphyria becoming manifest, cholestatic jaundice, rise in blood pressure in susceptible individuals, mental depression, cystitis-like syndrome, loss of scalp hair, erythema nodosum, hemorrhagic eruption, premenstrual-like syndrome, changes in libido, changes in appetite, nervousness, dizziness, fatigue, backache, erythema multiforme, itching, possible diminution in lactation when given immediately post-partum, irritability, malaise.

Dosage and Administration
Dosage requirements will vary with the severity of menopausal symptoms. Initially, doses ranging from 1.25 mg to 3.75 mg daily may be required to bring symptoms under control. When this has been accomplished dosage should then be gradually decreased until the minimal effective maintenance dose is established. This will usually be 0.625 mg or 1.25 mg daily or possibly less.

Caution
Federal law prohibits dispensing without a prescription.

How Supplied—0.625 mg tablets—bottles of 100 and 500
1.25 mg tablets—bottles of 100 and 500
2.5 mg tablets—bottles of 100



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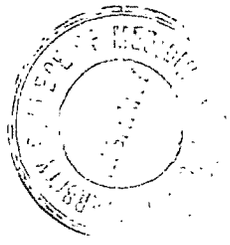
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GYNECOLOGY

Outpatient postconceptional fertility control with vaginally administered 15(S)15-methyl-$PGF_{2\alpha}$-methyl ester

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Stockholm, Sweden



15(S)15-methyl-$PGF_{2\alpha}$-methyl ester was administered as vaginal suppositories to terminate very early gestation in 16 outpatients who were thought to be pregnant. All patients received between 2.5 and 5.0 mg. of the prostaglandin compound. Pregnancy was confirmed later in 10 of the women. In these patients vaginal bleeding started 3 to 6 hours after the start of therapy and continued for an average of 8 days. By subjective assessment, the amount of bleeding during this time was equal to or slightly heavier than that of a normal menstruation for each woman. Patients were followed for 2 to 4 weeks with serial measurements of serum progesterone and HCG. There were no failures at early pregnancy interruption. Gastrointestinal side effects rarely were encountered; no serious complications occurred and no curettage had to be performed because of heavy bleeding. The vaginal use of the methyl ester of 15(S)15-methyl-$PGF_{2\alpha}$ for the termination of early pregnancy seems promising as a reliable and practical outpatient procedure.

From the Department of Obstetrics and Gynecology, Karolinska Sjukhuset.

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THE USE of prostaglandins to terminate very early gestation has been actively investigated as an acceptable nonsurgical method of fertility control. Of the many routes of administration studied, only the vaginal method offers the potential of self-administration of the compound that would make the therapy clinically useful on a large scale. The intrauterine instillation of $PGF_{2\alpha}$ as originally reported by Csapo and associates[1, 2] has produced the best results to date, but the technique requires heavy sedation, is suitable neither for self-administration nor for wide-spread clinical use,

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Table I. Results of vaginal administration of 15(S)-15-methyl-$PGF_{2\alpha}$-methyl ester

Case no.	*No. of days since LMP*	*Total prostaglandin dosage (mg.)*	*Onset of bleeding (hr.)*	*Duration of bleeding (days)*	*Plasma HCG (m.IU./ml.)*		
					Before therapy	*8 hr. into therapy*	*2 wk. after therapy*
1	39	2.5	10	6	2,640	2,100	2.7
2	31	2.5	48	4	4.4	4.0	2.9
3	33	2.5	6	7	4.5	3.0	—
4	35	3.5	8	11	6,000	—	2.6
5	36	3.5	5.5	5.5	155	135	8.8
6	35	3	4	6	2,400	1,650	47.5
7	37	3	6	6	1,100	980	15.5
8	35	3	3	6	3,000	2,120	8.6
9	37	4	3	6	9.2	5.5	8.8
10	41	4	3	9	2.3	3.4	5.4
11	41	4	4	10	9,300	6,320	42.3
12	39	3	5	9	2,300	940	11.5
13	43	3	3	13	17,600	9,200	28.0
14	37	4	8	4	4.4	6.6	8.6
15	35	4	6	9	17.5	11.5	6.1
16	42	5	8	10	2,660	1,820	8.5

and can be associated with severe adverse reactions.[3] Furthermore, of the prostaglandin compounds tested, natural E_2 and $F_{2\alpha}$ have never been uniformly consistent nor sufficiently specific to uterine rather than gastrointestinal musculature when administered in therapeutically effective amounts by the vaginal route.[4] Therefore, there have been concentrated efforts to find a suitable prostaglandin analogue that would increase the efficiency and efficacy of vaginal administration while minimizing the side effects.

This study was undertaken to determine if vaginally administered 15(S)15-methyl-$PGF_{2\alpha}$-methyl ester suppositories were effective early abortifacients up to 2 weeks following a first missed menstrual period.

Patients and methods

Sixteen healthy women varying in age from 19 to 43 years volunteered for therapy. The last menstrual period of these subjects ranged from 31 to 43 days prior to the time of treatment. A clinical diagnosis of very early pregnancy was confirmed later in 10 patients by the radioimmunoassay of serum HCG and progesterone.

Therapy was performed on an outpatient basis for up to 9 hours. Suppositories containing 0.5 or 1.0 mg. of 15(S)15-methyl-$PGF_{2\alpha}$-methyl ester were inserted vaginally at 3 hour intervals while the patients were observed for side effects. Oral Retardin (5 mg. of diphenoxylate chloride) and a Torecan rectal suppository (6.5 mg. of thiethylparazine) were administered routinely one half hour before each prostaglandin treatment. When a fourth or fifth vaginal suppository was given, it was administered by the patient upon her return home. Patients were seen at weekly intervals for 2 to 4 weeks to assess the efficacy of menses induction or pregnancy termination. All patients were followed until the serum HCG titer was less than 50 mI.U. per milliliter and examined 14 days after treatment to ascertain that the uterus had regressed to normal size.

Blood sampling for HCG and progesterone was performed at the beginning of therapy and 2, 4, 6, and 8 hours thereafter as well as after 1 and 2 weeks. Blood specimens were centrifuged within 10 minutes after sampling and 2 × 0.2 ml was immediately subjected to the HCG determination. Serum was then stored at −20° C. for later analysis.

HCG was determined with a commercially available kit (Serono, Italy) as recommended, with the exception that the soluble antibody was exchanged for a cellulose-bound rabbit antiserum (DASP, Organon, Holland) as originally suggested by Midgeley and described by den Hollander and Schuurs.[5] Into each assay tube was placed 0.2 ml. of the DASP suspension. A level of HCG exceeding 100 mI.U. per milliliter of serum was accepted as diagnostic of pregnancy.

Progesterone in 1.0 ml. of serum was assayed (according to the principles outlined by Midgeley and Niswender) with kits purchased from Micromedics (Philadelphia, Pennsylvania), used as recommended by the manufacturer. Due to the cross-reaction primarily between LH and HCG, a serum found to contain less than 300 mI.U. per milliliter of HCG was analyzed also with a comparatively specific radioimmunoassay for LH (Kabi, Stockholm, Sweden) which is about 10 times less sensitive to HCG. When comparatively low LH

Table II. Serum progesterone levels (ng./ml.) before and after vaginal administration of 15(S)15-methyl-$PGF_{2\alpha}$-methyl ester

Patient group	*Hours during therapy*					*Days after therapy*	
	0	*2*	*4*	*6*	*8*	*7*	*14-21*
Nonpregnant	7.5	6.2	4.6	5.3	7.1	0.6	0.7
Pregnant	24.5	24.0	23.6	21.3	18.1	5.4	1.4

values were obtained in this analysis it was regarded as a confirmation of pregnancy.

Results

Of the 16 patients treated, six of the group were not pregnant at the time of prostaglandin therapy. Details of treatment, laboratory data, and trial outcome for each patient are outlined in Table I. Vaginal insertion of 2.5 to 5.0 mg. of 15(S)15-methyl-$PGF_{2\alpha}$-methyl ester terminated 100 per cent of the early pregnancies. During the first 9 hours of prostaglandin therapy, 83 per cent of the nonpregnant and 90 per cent of the pregnant patients started uterine bleeding. The average duration of bleeding for those who were not pregnant was 6.5 days; mean duration for the pregnant patients was approximately 8 days. In no case was bleeding sufficiently heavy to indicate curettage. Most patients characterized the bleeding as similar in amount to a normal menstrual period with the heaviest flow occurring on the third or fourth day.

Laboratory analysis of serial blood samples drawn from the successfully treated pregnant patients during the first 8 hours of prostaglandin therapy demonstrated a slightly erratic but progressive decline in HCG and progesterone serum concentrations (Tables I and II). In this time interval mean percentage declines of 59 and 26 per cent were observed for HCG and progesterone levels, respectively. Normal nonpregnant values were achieved usually within 1 week. It is interesting that a transient fall in progesterone values did occur in several of the nonpregnant patients approximately 4 hours after the start of therapy. However, neither this trend nor the 8 hour 26 per cent decline in pregnant progesterone values were found to be statistically significant ($p > 0.05$). On the other hand, the fall in HCG was significant ($p < 0.05$). Blood sampling beyond the eighth hour of therapy and in a larger number of patients hopefully will further elucidate these trends.

Episodes of nausea, vomiting, or diarrhea were minimal. Twelve patients experienced no side effects whatsoever. Those that occurred in the other four patients were relatively mild and easily treated. Except for an episode of moderate pain experienced by one patient, discomfort was usually minimal and described as menstrual-like in nature.

Comment

The interruption of pregnancy during the postconceptional phase by a pharmacologic agent has been an attractive possibility for fertility control for some time. The vaginal use of 15(S)15-methyl-$PGF_{2\alpha}$-methyl ester shows promise as a nonsurgical, postconceptional abortifacient in early pregnancy for women who fail to use other methods of contraception or whose primary contraceptive method has failed. In the form and dosage described in this small study, there were no failures at early pregnancy interruption. Not only was the success rate satisfactory but side effects were minimal and certainly clinically acceptable.

The mechanism by which prostaglandin compounds effect early abortion is thought to be due mainly to increased uterine myometrial activity causing a disruption of the implanted ovum with resultant uterine bleeding.[6] It is not felt that luteolysis plays a major role. Our findings, which show that the decline in plasma progesterone follows a fall in HCG, are consistent with this hypothesis. Further investigation of this probably complex phenomenon is under way by our group.

The uterine potency of 15(S)15-methyl-$PGF_{2\alpha}$-methyl ester combined with its relatively fewer gastrointestinal side effects than those seen with the natural prostaglandins make this compound a promising agent for postconceptional fertility control. Its use in the form of vaginal suppositories is acceptable to patients, suitable for outpatient management, and, once enough experience has been gained, it may be suitable for self-administration under medical supervision.

The authors wish to express their sincere thanks to the Clinical Research Unit nursing staff for their valuable assistance.

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VIth International Seminar on Reproductive Physiology and Sexual Endocrinology

The VIth International Seminar on Reproductive Physiology and Sexual Endocrinology will be held at the Free University of Brussels, Brussels, Belgium, May 31 to June 2, 1976. Members of the Organizing Committee are: P. O. Hubinont, L. Martini, P. Franchimont, C. Robyn, and M. L'Hermite. Invited lectures on biological and clinical aspects will be presented by: G. M. Besser, G. Copinschi, M. Ferin, J. Flament-Durand, P. Franchimont, K. Fuxe, M. Hamon, F. Labrie, M. L'Hermite, S. M. McCann, R. M. MacLeod, L. Martini, J. Mendlewicz, J. L. Pasteels, J. P. Raynaud, C. Robyn, H. P. G. Schneider, H.-D. Taubert, and S. S. C. Yen. Some of the topics to be discussed include: neurotransmitters, hypothalamic hypophysiotropic hormones, somatostatin, prolactin, and neuroendocrine physiopathology and pharmacology, with the emphasis to be on reproduction. Free communications are invited, with abstracts to be submitted on special forms by April 15, 1976. Priority for oral presentations will be given to papers dealing with clinical studies.

The deadline for advance registration and abstract submission is April 15, 1976. For further information, contact: Mrs. P. Jonart, Service de Gynécologie, Hôpital Saint-Pierre, Rue Haute 322, B 1000 Bruxelles, Belgium.

Contraceptive knowledge and practice among undergraduates at a Canadian university

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A total of 509 undergraduate students, aged 17 to 23 years, were tested to assess the adequacy of their knowledge of methods of birth control. A level of adequate knowledge was arbitrarily defined a priori as one standard deviation below the mean score of a random sample of second-year medical students, who were assumed to have more than adequate knowledge on the basis of prior courses in reproductive physiology and human sexuality. Of the undergraduate population sampled, 34 per cent were found to have inadequate knowledge. This population is in need of birth control information. Of nonvirgins in the sample, 20.2 per cent risk unwanted pregnancy, the majority (78.3 per cent) in spite of adequate knowledge. This suggests that lack of motivation, rather than purely lack of information, is an important factor contributing to the unwanted pregnancy rate of the college student population.

THREE TYPES of studies suggest that lack of information about birth control is a serious problem among young people in general and among college students in particular: the unwanted pregnancy rates, surveys of birth control use, and surveys of birth control knowledge.

To the extent that illegitimate is equivalent to unwanted or unplanned, current illegitimacy rates may be taken as evidence of lack of use of contraceptives, which may be due to lack of knowledge. Fujita and associates[1] estimated that one of every 11 children are illegitimate (this figure agrees well with Canadian 1971 D.B.S. results,[2] and that one out of six women gives birth within 8 months after her first marriage. When the mother is in the 15 to 17 or 18 to 19 year age range, the proportion of illegitimate children rises to two fifths and one fifth, respectively; over half the first-born children of girls aged 15 to 19 were conceived prior to marriage.[3] Among unmarried female college students, McCance and Hall[4] found that 10 per cent of nonvirgins had had an unwanted pregnancy.[4]

Coe and Blum,[5] in fact, showed that the rate of pregnancies to unmarried college students not only stopped rising but actually declined after a program providing information and counselling on birth control was established by the university. Others point to the abortion rates of teenagers and young adults as an indication that sexual education and contraceptive counselling are urgently required.[6]

Reports on use of birth control among college students suggest that a disturbingly high percentage of students risk unwanted pregnancy. In Lundy's[7] study of unmarried female college students, only 45 per cent of sexually active subjects said that they used a method of birth control. Fujita and associates found, among

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unmarried undergraduates, that 54 per cent of males and 46 per cent of females failed to use some form of contraception on every occasion of intercourse. Of those who did attempt birth control, 14 per cent of males and 40 per cent of females depended on withdrawal (which has a clinical failure rate of 20 to 30 per 100 woman-years of exposure,[8] and 13 per cent of males and 31 per cent of females used rhythm (which has a failure rate of 15 to 30 per 100 woman-years of exposure[8].[1] Studies by McCance[4] and Bauman[9] revealed a similar extent of exposure to unwanted pregnancy, and emphasize the first occasion of sexual intercourse as being particularly likely to occur without use of contraception. To what extent is this ineffective contraceptive behavior due to inadequate knowledge?

Direct assessments of the state of undergraduates' sexual knowledge range from the impression of Sarrel and Sarrel,[10] based on clinical experience, of "widespread ignorance about sex" to Fujita's[1] report of "over-all understanding of the facts," based on the results of a written questionnaire on basic sexual physiology and contraception. Even the more favorable reports, however, found striking gaps in the extent of sexual knowledge, e.g., students had unrealistic perceptions of pregnancy risk[1] and half or less of sexually active young people were aware of the correct relationship of greatest risk of pregnancy to the menstrual cycle.[11–13]

The aims of the present study were to survey the contraceptive knowledge and practices of undergraduate students at a large Canadian university. In general, we were interested in obtaining a Canadian perspective on sexual activity of college students and on the problems of contraceptive negligence and its sequelae. In particular, we wished to answer the following questions: (1) Do people who have engaged in sexual intercourse have what we consider to be adequate knowledge of contraception? (2) Do those whom we consider adequately informed tend in practice to risk unwanted pregnancy in spite of sufficient knowledge? (3) What sources are most effective in providing adequate knowledge about birth control?

Focus on knowledge as a major determinant of birth control use does not exclude consideration of the contributions of motivation and accessibility factors, but rather helps to clarify the relative importance of these factors.

The study

The sample. Two groups of students were involved in the study, the pretest sample group and the study sample group itself. The data collected from the pretest group were used in the evaluation of the grading system and in the formulation of a definition of adequate knowledge.

The pretest sample consisted of 100 medical students randomly selected from the second-year class. Of these, 99 responded to our questionnaire. We chose the second-year medical class on the assumption that these students would have more than adequate knowledge about contraception since, at this particular university, they have been exposed to considerable information about birth control in the course of lectures on reproductive physiology and on obstetrics and gynecology, and seminars on human sexuality; unlike their British counterparts, they have also had 4 or 5 years of prior university experience. Arbitrarily, the level of adequate knowledge was defined beforehand as one standard deviation below the mean score of the pretest sample. Since the mean score of the pretest sample was 35.6 (±5.9 S.D.) out of a possible score of 43, adequate knowledge was defined as a score of 29.7 or above.

The study sample consisted of 509 students aged 17 to 23 studying Child Psychology or Psychology Statistics. These classes were chosen because they were large in size and contained students with diversified college backgrounds (i.e., arts, science, commerce, engineering, and religion). Questionnaires were distributed at the beginning of the lecture and were collected 15 minutes later (before the lecture started). Of the 616 responses, 107 were rejected. Seventy were deleted because the answer cards were filled out incorrectly (e.g., two responses for one question); the remainder did not fall into the predefined age distribution (17 to 23).

The questionnaire. The knowledge questions were restricted to concepts directly applicable to the prevention of pregnancy and were graded according to difficulty and saliency of information. There were five items on basic knowledge of human reproductive physiology (graded at 3 points each), three items on the relative effectiveness of different methods of birth control (graded at 6 points each for a correct answer and 2 points for a partially correct answer), and five items dealing with information essential to the successful contraceptive use of specific methods (graded at 2 points each). A point biserial correlation between total score and the responses to individual questions showed all of the questions to be valid discriminators between knowledgeable and unknowledgeable subjects.

Eight remaining items dealt with the sample characteristics in which we were interested: age, sex, major source of information about birth control, subject's assessment of his own knowledge, whether the subject

had ever had sexual intercourse, method of contraception used most frequently by subject or coital partner, whether subject or coital partner had ever had an unwanted pregnancy, and prevalence of "risk-taking."

Results

Characteristics of the sample population. The sample consisted of 509 undergraduates; 24.2 per cent of these were male and 75.8 per cent were female. Ages ranged from 17 to 23 years inclusive, with mean age being 19.4.

There were 228 students (44.8 per cent of the sample) who were sexually active, i.e., had experienced sexual intercourse at one time or another; 57.7 per cent of males were nonvirgins, compared to 40.7 per cent of females. Virgins were in the majority in each of the age groups 17, 18, and 19 years; nonvirgins were in the majority in each of the older age groups (except among the small population of 22-year-olds, where virgins are in a slight majority).

Findings in knowledge and score. The mean score of the study group was 31.4 (± 0.3 S.E. = standard error), which is lower than that of the pretest students (as expected) but above the predefined level of adequate knowledge.

No marked differences in knowledge emerged between males and females: similar proportions of each had adequate knowledge (66.7 per cent of males, 65.8 per cent of females), and their mean scores were virtually identical (31.5 ± 0.5 S.E. and 31.4 ± 0.4 S.E., respectively). Every age group had adequate knowledge but, surprisingly, there was no improvement in score with age.

Of sexually active students, 79.8 per cent were found to have adequate knowledge, compared to only 54.8 per cent of virgins, which suggests that the people who most need contraceptive information (i.e., sexually active students) are more likely to actually have it. Female nonvirgins had the highest mean score (34.4 ± 0.5 S.E.) probably because they have the greatest incentive to acquire adequate knowledge and avoid pregnancy, followed by male nonvirgins (32.7 ± 0.7 S.E.) and male virgins (29.8 ± 0.9 S.E.). The mean score of female virgins (29.4 ± 0.5 S.E.) puts this group into the inadequate knowledge category; for this group, the incentive to acquire contraceptive information may be outweighed by the social sanctions against expressing interest in premarital sex.[1, 4, 9, 14]

There were 240 students who thought their knowledge of contraception was inadequate; in 52.1 per cent of cases this agreed with our evaluation of their knowledge (they scored below 29.7 on the questionnaire). Another 269 students thought they had adequate knowledge of contraception; of these, 82.2 per cent did have adequate knowledge by our standards and the 17.8 per cent who did not have adequate knowledge but thought they did are presumably at high risk for inadvertently causing an unwanted pregnancy.*

Trends in source of information about contraception are shown in Table I. Each source—except "friends and lovers"—provided adequate information to the majority of students using that source.

Table I. Complete sample, major source of information on contraception

Major source of information on contraception	*Total no. of students*	*Per cent of students*	*Per cent of students with adequate knowledge*
Printed matter (booklets, pamphlets, etc.)	373	73.3	67.6
Parents	12	2.4	66.7
Friends and lovers	68	13.4	48.5
Sex education course, school, or other educational institution	25	4.9	72.0
Doctor	31	6.1	80.6

Table II. Trends in contraceptive use

Method of contraception used most frequently by student or coital partner	*All nonvirgins*		*Males*	*Females*
	No. using method	*% using method*	*% using method*	*% using method*
Rhythm	5	2.2	2.8	1.9
Postcoital douche	1	0.4	0.0	0.6
Pill	143	62.7	62.0	63.1
Condom	54	23.7	25.4	22.9
IUD	4	1.8	0.0	2.5
Diaphragm and jelly	5	2.2	0.0	3.2
Vaginal spermicides	1	0.4	0.0	0.6
Vasectomy or tubal ligation	0	0.0	0.0	0.0
Coitus interruptus	12	5.3	7.0	4.5
Pot luck	3	1.3	2.8	0.6

*Proportionately, more students underestimated than overestimated their knowledge by our standards. This suggests two interesting considerations: (1) our standards were too low or did not test some essential areas of knowledge, or (2) people are unwilling to admit to knowing a lot about contraception, perhaps because it would suggest that they are interested in premarital sexual activity of which peers, parents, and society in general are thought to disapprove and because it would imply that they know enough to take responsible birth control action in sexual encounters, a conclusion that some students may wish to avoid. In this latter regard, it is interesting to note that a higher percentage of the males than of the females underestimated their level of knowledge!

Findings in risk-taking and its consequences. Table II shows trends in use of birth control. Only 1.3 per cent of sexually active students said that they regularly used no method of contraception. However, 20.2 per cent of nonvirgins currently risk unwanted pregnancy. Risk taking was assessed by asking, "Currently, do you *ever* engage in sexual intercourse without using *any one* of the following contraceptive techniques—the pill, intrauterine device, the condom, diaphragm and jelly?" A "yes" response to this question was defined as risking unwanted pregnancy. The methods listed were chosen because they are reported to have a clinical failure rate of 20 or less per 100 woman-years of exposure.[8]

Of the nonvirgin sample, 9.6 per cent reported that they had been involved with an unwanted pregnancy. Since 11.5 per cent of women students but only 5.6 per cent of males reported involvement with unwanted pregnancies, one might suspect under-reporting by males due to lack of awareness or belief in the pregnancies of girlfriends whom they had not continued to see. Interestingly, the mean score of the unwanted pregnancy group was 32.6 (± 1.4 S.E.), well above the adequacy level. However, 27.3 per cent of these students still had inadequate knowledge of birth control, and 27.3 per cent still took risks.

Comment

Of those students who have engaged in sexual intercourse, 20.2 per cent have inadequate knowledge of contraception by our criteria and a similar proportion (20.2 per cent) risk unwanted pregnancy by failing to use an effective method of birth control. Are these the same people? While the mean score of risk takers (33.5 ± 0.8 S.E.) was lower than that of non–risk takers (34.0 ± 0.5 S.E.), it was nonetheless well above the level of adequate knowledge. In fact, 78.3 per cent of risk takers scored above the level of adequate knowledge. This suggests that adequate contraceptive knowledge does not necessarily give rise to adequate contraceptive behavior; intervening factors such as motivation and accessibility play an important part in the risk taking and subsequent unwanted pregnancies of young adults.

Some students may use a very effective method of contraception but be abysmally ignorant in terms of their score on the questionnaire. This may reflect the occurrence of social compliance, where a person ignorant about contraception accepts and acts upon the advice of a person (e.g., a physician) designated by society as an expert in this field. Our study was not intended to discover whether students were socially compliant, but rather to determine whether students possessed the basis for cognitive consensus, i.e., whether they themselves knew enough about differences in contraceptive effectiveness to be able to accept (or reject) expert advice on the basis of their own knowledge.

How does our Canadian student population compare with United States college populations? The percentages of sexually active male (57.7 per cent) and female (40.7 per cent) students are very similar to U. S. estimates, which range from 45 per cent[1] to 60 per cent[14] for males and from 30.5 per cent[1] to 47 per cent[7] for females. The age at which the population changes from virgin to nonvirgin majority (between ages 19 and 20) agrees well with the findings of Kantner and Zelnick.[13]

In two areas however, our findings differ markedly from those of U. S. studies. The incidence of risk taking was much lower in our student sample: 90.4 per cent of sexually active students usually used an effective method of contraception and 79.8 per cent always used an effective method of contraception. This contrasts sharply with the findings of Lundy[7] and Fujita and associates[1] cited earlier, in which half or more of students failed to use any form of contraception, effective or otherwise. Second, a striking 73.3 per cent of our Canadian sample said that printed matter was their chief source of information on birth control, with only 13.4 per cent of students relying on peers. Thornburg,[15] in agreement with earlier U. S. studies by Ramsey and Bell, found peers to be the most popular chief source of information, used by 37.9 per cent of his sample, with literature accounting for only 20.6 per cent (barely ahead of mothers, 19.3 per cent, and schools, 14.8 per cent). Even for sophomore medical students in the United States, peers were the main source of information for a majority (57.4 per cent of students.[16]

The popularity of printed matter may reflect the particular situation at this univeristy, where a comprehensive and detailed birth control handbook has been widely distributed. Nonetheless, it is a little disappointing to note that only 67.6 per cent of students using printed matter have adequate knowledge, parents being just as effective a source of information. This may suggest the need for a more simple and direct guide to the essentials of birth control, perhaps a summary of the very thorough birth control handbook. An alternative interpretation, however, and one that is less easily remedied, must also be considered: regardless of the free availability of excellent printed materials on birth control, if people are not motivated to retain or use this information the impact of such materials on their knowledge and usage

of contraception is bound to be minimal. For this group of students, the decision to have intercourse without effective contraception (similar perhaps to the decision to continue smoking or to drive without using automobile seat belts) may be based on factors other than rational or objective considerations. The "unexpected," unplanned nature of intercourse, and the overwhelming desires of the moment, conflict with the requirement of considerable forethought for use of most methods of birth control.[4, 9]

The results of Table II further suggest that the doctor, when used, is a highly effective source of adequate knowledge, emphasizing the need for the physician, especially the campus physician, to take a more active role in birth control education. A university campus of over 15,000 people may be likened to a small city with the important difference that virtually all of its citizens are of a potentially fertile and sexually active age. A more visible univeristy involvement in contraception, e.g., the establishment of a central, easily accessible, and emotionally supportive birth control clinic (as at Yale[10]) might go far toward improving student willingness to use contraception, as well as providing reliable information and advice.

To summarize the chief findings of the study, 34 per cent of the undergraduates sampled were found to have inadequate knowledge. This segment of the population does need more information about birth control but, by far the majority of the students who risk unwanted pregnancy did so in spite of adequate knowledge. This emphasizes that motivation, rather than purely lack of information, plays an important role in the unwanted pregnancy rate of the college student population.

We are indebted to Drs. J. G. Lohrenz and R. A. Kinch for advice and encouragement, and to Drs. S. Shapiro, I. Simon, B. Kaplan, and R. Hutcheon for critical reviews.

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Appendix: The questionnaire

The Medical Students at McGill University would like your assistance by answering the following questions on contraception. The success of this project depends highly on the integrity of your responses, so *please follow the instructions carefully.*

DO *NOT* PUT YOUR NAME, STUDENT NUMBER, OR ANY OTHER FORM OF IDENTIFICATION ON YOUR IBM CARD. We are not interested, *nor* will we be able, or try to trace your answers back to you.

SAMPLE QUESTION

1. The sky is: (1) blue
   (2) black
   (3) red
   (4) grey
   (5) blue during a clear day, black at night, red with the sunset, grey when it is cloudy.

—answer the *most* appropriate answer on the accompanying IBM card—*only with the special pencil*

provided—fill in the space completely without going outside the boundaries.

—best answer to sample question is [5]

(1) [1] [2] [3] [4] ■
(2)
(3)
(4)
etc.

—if you must erase, erase completely so that only *ONE* answer remains—please do *not* bend or mutilate your computer card.

PLEASE FILL IN *ONE ANSWER* ON THE IBM CARD PROVIDED FOR THE FOLLOWING QUESTIONS (WITHOUT HELP FROM OTHER SOURCES)

Your age as of September 1, 1973 is (choose one from numbers 1 or 2)

1. (1) 15 or under
   (2) 16
   (3) 17
   (4) 18
   (5) 19
2. (1) 20
   (2) 21
   (3) 22
   (4) 23
   (5) 24 or more

Your sex is: (1) male
(2) female

4. A woman who has intercourse, is *most likely* to become pregnant if intercourse occurs:
   (1) Just before menstruation
   (2) during menstruation
   (3) right after menstruation
   (4) 2 weeks before menstruation
5. Before orgasm there can be a few drops of semen which escape (orgasm) = climax; semen = seminal fluid, fluid containing sperm)
   (1) true
   (2) false
   (3) don't know
6. *One* drop of semen (seminal fluid, i.e., fluid containing sperm) is enough to fertilize an ovum (i.e., cause pregnancy):
   (1) true
   (2) false
   (3) don't know
7. For the incidental sexual encounter, the *best* form of contraception is:
   (1) the condom (safe)
   (2) spermicidal foam or jelly (spermicidal means "kills sperm")
   (3) postcoital douche (postcoital = after intercourse, douche = washing)
   (4) coitus interruptus (withdrawal before orgasm)
   (5) hot bath preceding intercourse
8. Which of the following arrangements represents modes of contraception from *most* to *least* effective in preventing pregnancy?
   (1) condom (safe), pill, rhythm
   (2) rhythm, pill, condom
   (3) condom, rhythm, pill
   (4) pill, condom, rhythm
   (5) pill, rhythm, condom
9. For a woman on oral contraceptives to become pregnant, the *least* number of days she would have to miss taking the pill is:
   (1) for 2 consecutive days in a normal menstrual cycle
   (2) for 7 consecutive days in a normal menstrual cycle
   (3) for 14 consecutive days in a normal menstrual cycle
   (4) for more than half the days of a normal menstrual cycle regardless of which days
   (5) for the whole length of a normal menstrual cycle
10. Vaseline is a good lubricant for condoms:
    (1) true
    (2) false
    (3) don't know
11. Condoms tend to tear more easily if kept in hot, moist places, like wallets and pockets:
    (1) true
    (2) false
    (3) don't know
12. The diaphragm can be removed 2 hours following intercourse and still give very good contraceptive protection:
    (1) true
    (2) false
    (3) don't know
13. Sperm cells, if deposited between the labia majora (vaginal lips), can result in pregnancy, even in a virgin with an intact hymen (protective membrane over vaginal orifice):
    (1) true
    (2) false
    (3) don't know

14. A woman can avoid becoming pregnant if she does not achieve an orgasm:
    (1) true
    (2) false
    (3) don't know

15. Which of the following arrangements represents the modes of contraception from *most* to *least* efficient?
    (1) IUD (intrauterine device), diaphragm, Coitus interruptus (withdrawal)
    (2) IUD, coitus interruptus, diaphragm,
    (3) diaphragm, coitus interruptus, IUD
    (5) coitus interruptus, IUD, diaphragm

16. The postcoital douche is an effective means of contraception (postcoital = after intercourse, douche = washing):
    (1) true
    (2) false
    (3) don't know

17. Do you consider that you have adequate knowledge of birth control?
    (1) yes
    (2) no

18. Which of the following categories represents your *major* source of information on contraception?
    (1) printed information (e.g., booklets, magazines, etc.)
    (2) parents
    (3) friends and lovers
    (4) a sex education course, school or other educational institution
    (5) your doctor

19. Have you engaged in sexual intercourse?
    (1) yes
    (2) no

IF YOUR ANSWER WAS "YES" TO THE PREVIOUS QUESTION, THEN PLEASE REPLY TO NUMBERS 20 TO 23.

IF YOUR ANSWER WAS "NO," THEN LEAVE THE REMAINING QUESTIONS *BLANK*.

Choose *only one* method of contraception (from Numbers 20, 21, and 22) that you, or your partner use *most frequently*.

20. (1) rhythm method
    (2) postcoital douche
    (3) the pill
    (4) condom (safe)
    (5) I use a contraceptive technique listed in No. 21 and No. 22 *most frequently*

21. (1) IUD (intrauterine device)
    (2) diaphragm and jelly
    (3) vaginal spermicides
    (4) vasectomy or tubal ligation
    (5) I use a contraceptive technique listed in No. 20 and No. 22 *most frequently*

22. (1) coitus interruptus (withdrawal)
    (2) pot-luck
    (3) I use a contraceptive technique listed in No. 20 and No. 21 *most frequently*

23. Have you or a partner in sexual intercourse ever had an unwanted pregnancy?
    (1) yes
    (2) no

24. Currently, do you *ever* engage in sexual intercourse without using *any one* of the following contraceptive techniques?
    —the pill
    —intra-uterine device
    —the condom
    —diaphragm and jelly
    (1) yes
    (2) no

ANSWERS WILL BE POSTED TOMORROW FOR THOSE WHO ARE INTERESTED. THANK YOU FOR YOUR COOPERATION.

Inhibition of spontaneous contractility of the human and the rabbit Fallopian tube by potassium

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Spontaneous contractions of circular and longitudinal muscle of the ampulla and isthmus of human and rabbit Fallopian tubes were recorded isometrically. Addition of K^+ to tissue in K^+-free medium always caused immediate inhibition of spontaneous contractility. K^+-induced inhibition was prevented by ouabain and reversed by removal of K^+. It is suggested that K^+ caused inhibition of contractility by activating electrogenic Na^+ pumping with resultant hyperpolarization of the muscle cells.

CONSIDERABLE EVIDENCE exists that the sodium pump in many tissues is electrogenic as a result of unequal coupling between Na^+ extrusion and K^+ accumulation.[1, 2] The Na^+ pump determines the resting membrane potential in many smooth muscles by maintaining a diffusion potential and by producing an electrogenic potential.[2] Activation of Na^+ pumping causes inhibition of the spontaneous contractility of several smooth muscles,[2] including myometrium,[3, 4] as a consequence of membrane hyperpolarization resulting from the electrogenic extrusion of Na^+.

The delay in ovum transport occurring at the ampullary-isthmic junction and in the isthmus of human beings and other mammals[5] is generally considered to result from a functional block in this region of the Fallopian tube. Such a block could result from increased sympathetic activity[6] or altered contractility of the tube. The present study is part of a continuing study of the myogenic control systems in the Fallopian tube.[7, 8] This study was undertaken in order to demonstrate the existence of an electrogenic Na^+ pump in the smooth muscle of both the human and the rabbit Fallopian tube since such a pump could modify contractility and be hormonally dependent.

The method used in this study was essentially similar to that used previously in this laboratory to demonstrate electrogenic Na^+ pumping in rat and rabbit myometrium[3, 4] and in rabbit detrusor.[9] It is based on the finding that the contribution electrogenic Na^+ pumping makes to the membrane potential is particularly significant when tissues have gained Na^+ and are subsequently allowed to recover when K^+ is returned to the medium.[1] Thus, in the present study, as in the earlier studies of rabbit myometrium[4] and detrusor,[9] tissues were exposed to K^+-free medium for at least 30 minutes in order to allow partial downhill ion movements (i.e., gain of Na^+ and loss of K^+) to occur as a result of inhibition of Na^+ pumping. K^+ was then added to the medium in order to activate Na^+ pumping, and the accompanying changes in contractility were monitored.

Material and methods

Portions of human Fallopian tube were obtained at tubal ligation and hysterectomy and were immeidately placed in Krebs solution of the follcwing composition (millimolar): NaCl, 115.5; KCl, 5.4; $CaCl_2$, 2.5; $MgCl_2$, 1.2; NaH_2PO_4, 1.2; $NaHCO_3$, 22.0; glucose, 49.2. The

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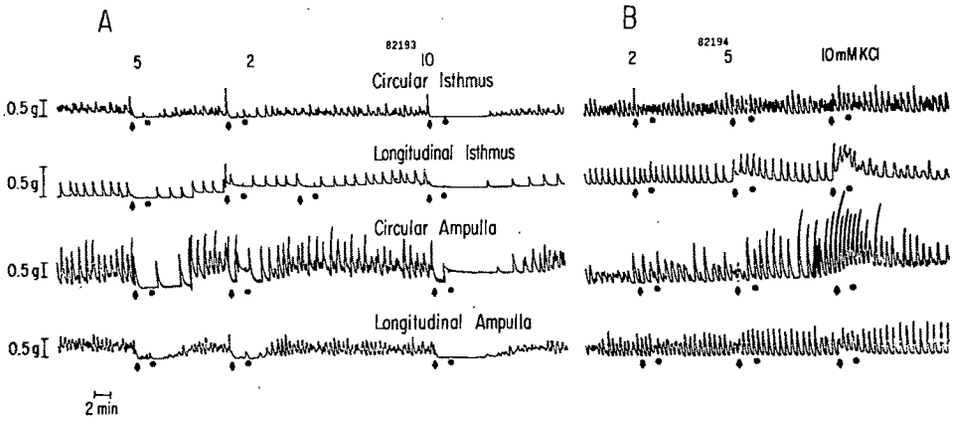


Fig. 1. Effect of KCl on spontaneously contracting rabbit Fallopian tube bathed in K^+-free Krebs solution. Circular and longitudinal preparations of isthmus and ampulla were exposed to media containing KCl in the concentrations indicated (at the arrow) and returned to K^+-free Krebs solution (at ●). In *B*, tissues were exposed to 10^{-5}M ouabain throughout.

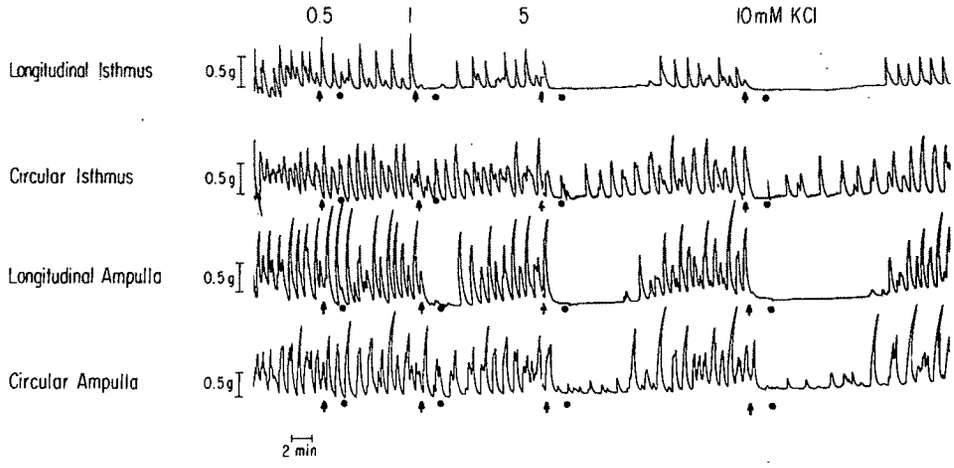


Fig. 2. Effect of KCl on spontaneously contracting human Fallopian tube in K^+-free Krebs solution. KCl added at arrow and removed at ●. Tissues removed at hysterectomy, secretory phase.

medium was bubbled with 95 per cent oxygen/5 per cent carbon dioxide. Human material was obtained from patients during the proliferative and secretory phases and during pregnancy.

Female New Zealand rabbits were injected with 100 μg of 17β-estradiol subcutaneously daily for at least six days prior to sacrifice by air embolism. The oviduct was removed and placed in the Krebs solution; the isthmus and ampulla were identified and dissected free of surrounding tissue.

From each Fallopian tube, cross-sectional segments of Fallopian tubes were cut and joined by thread in order to form a chain to allow recording of circular muscle activity. The remainder of each tube was left intact to allow recording of longitudinal muscle activity. Tissues were mounted in 5 ml. organ baths maintained at 26° C. and bathed in solution equilibrated with 95 per cent O_2/5 per cent CO_2. Contractions of both longitudinally and circularly orientated smooth muscle preparation were recorded isometrically with force displacement transducers* connected to a dynograph.† A resting tension of 0.5 Gm. was applied to all tissues.

The tissue was allowed to equilibrate for 30 minutes

*Model FT03C, Grass Instrument Co., 101 Old Colony Ave., Quincy, Massachusetts 02169.

†Beckman RB, Beckman Instruments, Inc., 2500 Harbor Blvd., Fullerton, California 92634.

before differing concentrations of K^+ were added to the bath from a 1.0M KCl stock solution. The medium bathing the tissue was then replaced by K^+-free Krebs solution, in which sucrose was substituted for K^+. After at least 30 minutes, the tissues were then again challenged with differing concentrations of K^+ in the presence and absence of ouabain (10^{-5}M). This format was used in studies made on 10 rabbit and 6 human Fallopian tubes.

Results

The addition of 5 to 20 mM. KCl to longitudinal and circular preparations of rabbit and human Fallopian tubes in Krebs solution (containing 5.4 mM. KCl), caused an increase in tension and/or an increase in the frequency of spontaneous contractions in all tissues.

When tissues were bathed in K^+-free Krebs solution, spontaneous contractions continued, and frequently their frequency was increased. The subsequent addition of KCl produced an inhibition of tension and/or of spontaneous muscular activity in all tissues studied. The effect of K^+ was concentration-dependent, less than 1 mM. being ineffective. When tissues were returned to K^+-free solution, this inhibition of muscular activity was reversed in all tissues studied. The time taken for contractions to return was related to the preceding K^+ concentration used, i.e., the higher the K^+ concentration, the longer the quiescent recovery period. A number of circular muscle preparations from both ampulla and isthmus were less sensitive to inhibition by K^+ and recovered their spontaneous activity more rapidly when K^+ was removed.

The effects of 10^{-5}M ouabain on the inhibition of motility produced by the addition of KCl were also examined. After tissues had been exposed to K^+-free Krebs solution, tissues were exposed to 10^{-5}M ouabain for 3 minutes before KCl was added. In all cases studied, the contractions of tissues treated with ouabain were not inhibited by the addition of KCl, but rather they responded as if they were in normal Krebs solution, i.e., there was an increase in spontaneous contractility and/or basal tension. The initial addition of ouabain itself frequently increased spontaneous muscular activity as has been noted in several other smooth muscle preparations.[2, 3, 11] Tissues were not exposed to ouabain for prolonged periods because, under these circumstances, marked downhill ion movements occur and spontaneous contractility ceases.[11]

These features are shown in Figs. 1 and 2 which illustrate and are representative of studies made on 10 rabbit and 6 human Fallopian tubes. In all these cases, addition of K^+ to tissues in K^+-free medium caused inhibition of contractility, and this effect was blocked by ouabain.

Comment

The main finding of this study was that addition of K^+ to tissues bathed in K^+-free medium always caused immediate inhibition of spontaneous contractility. The inhibition produced by addition of K^+ was always reversed by removing the potassium from the bathing medium and was always prevented by ouabain. Therefore, these characteristics are similar to those of the Na^+- and K^+-activated ATPase[10] and to those of electrogenic Na^+ pumps described in other smooth muscles.[2–4, 9] The observed inhibition of spontaneous activity of Fallopian tubes may have been caused by hyperpolarization of the smooth muscle cells due to unequal coupling between Na^+ extrusion and K^+ accumulation by the Na^+ pump.[1, 2] Proof that electrogenic Na^+ pumping is responsible for the inhibition observed will depend upon direct measurement of the membrane potential in order to compare the magnitude of the membrane potential observed with the calculated K^+ equilibrium potential.

Electrogenic Na^+ pumping in other smooth muscles (e.g., rat myometrium) has been found to be influenced by temperature in that the time course and magnitude of the resulting hyperpolarization were altered as the temperature was reduced.[3] However, as in the present study, electrogenic Na^+ pumping could still be demonstrated at 25 to 26° C. The present study was performed at 26° C. because spontaneous contractility was better maintained at this temperature than at 37° C.

The mechanism of regulation of ovum transport remains unexplained. Certainly the adrenergic innervation is not essential for either ovum transport[12, 13] or fertility.[14] The present finding suggests that alterations in the activity of the Na^+ pump in the smooth muscle of the rabbit and the human Fallopian tube can alter the membrane potential and hence the contractility of the tube. Whether such a mechanism is hormone-dependent and involved in the regulation of ovum transport is under further study in this laboratory.

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Carcinoma in situ of the vagina following treatment for carcinoma of the cervix: A distinctive clinical entity

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A series of 10 patients who presented with carcinoma in situ of the vagina following treatment for carcinoma of the cervix were seen in a 3 year period and their clinical and histologic features reviewed. Vaginal lesions developed 1 to 22 years after the diagnosis of carcinoma of the cervix was made. In three patients the vaginal lesions progressed to invasion. In the remaining patients repeated biopsies have revealed persistent carcinoma in situ. From this series of 10 patients we conclude that the biologic behavior of vaginal carcinoma in situ following carcinoma of the cervix resembles that of carcinoma in situ of the cervix and that carcinoma of the vagina following carcinoma of the cervix should be considered a separate clinical entity distinctive from primary invasive carcinoma of the vagina.

A SERIES of 10 patients who have been followed after treatment for carcinoma of the uterine cervix have developed abnormal cytologic and histologic findings in the vagina. These patients present a diagnostic puzzle and a problem in clinical management. The histologic changes in the vagina have varied from mild dysplasia to squamous-cell carcinoma, usually without invasion. Often the cellular atypia has been ascribed to previous therapy when it becomes known that the patient was treated for carcinoma of the cervix with surgery and/or radiotherapy.

There is no well-defined characterization of in situ vaginal carcinoma following treatment for other pelvic malignancies. Physicians following these patients show some reluctance to treat vaginal malignancy following therapy for other primary pelvic neoplasms with the same type of radical surgery or radiotherapy applied to the original carcinoma. This reluctance is based on insufficient information regarding the natural history of this disease, equivocation regarding the pathologic diagnosis, and the fact that the therapist has frequently expended most major therapeutic modalities against the original carcinoma. This report deals with a group of 10 patients followed from 2 to 7 years who presented with in situ vaginal carcinoma following treatment for carcinoma of the cervix. Since for various reasons the patients were not treated for the in situ vaginal carcinoma, we had the opportunity to observe the biologic behavior of this neoplasm.

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Case reports

Patient 1. L. S., a 55-year-old nulliparous woman, was admitted to the Surgery Branch of the National Cancer Institute (NCI) for evaluation of abnormal Papanicolaou (Pap) smears. Thirty months prior to her admission, she was hospitalized at another institution for a "Class V" Pap smear. Biopsies taken from an unspecified site at the vaginal apex revealed carcinoma in situ with several microscopic foci of early invasion. She was treated by total abdominal hysterectomy and right salpingo-oophorectomy. The resected specimen revealed squamous-cell carcinoma in situ in the cervix and epithelial atypia in the apex of the vagina.

Close follow-up examinations have shown cytopathologic diagnoses ranging from severe dysplasia to

frank squamous-cell carcinoma, and biopsies have shown histologic diagnoses ranging from advanced epithelial atypia to squamous-cell carcinoma in situ. She continues to be followed closely with the plan to institute treatment when invasive carcinoma can be confirmed. Biopsies in July, 1974, 4 years after admission to NIH, revealed squamous-cell carcinoma in situ.

Patient 2. J. W., a 46-year-old gravida 2, para 2 woman, in November of 1968 underwent radical abdominal hysterectomy and pelvic lymphadenectomy for a "Stage I" epidermoid carcinoma of the uterine cervix. One of four left iliac lymph nodes contained metastatic carcinoma. The resected vaginal cuff showed no evidence of carcinoma but revealed severe epithelial dysplasia. During the first 2 months postoperatively, the patient was treated with external-beam cobalt radiotherapy and received 6,022 r. to the midpelvic plane.

The patient was lost to follow-up until February, 1970, at which time a Pap smear was found to be consistent with invasive epidermoid carcinoma, and subsequent biopsies were reported as carcinoma in situ. Throughout the following year, the patient was followed closely, with several cytologic diagnoses of epidermoid carcinoma. Multiple biopsies of the vagina showed changes ranging from epithelial atypia to superficially invasive epidermoid carcinoma. When two foci of invasion were found in the vaginal biopsies in late 1970, the patient was advised to undergo exenterative surgery. The patient declined the operation and avoided follow-up appointments for the next 3 years despite repeated expressions of concern from her physicians. In March, 1973, she returned for evaluation because of postcoital bleeding of 2 months' duration. On physical examination, the vagina had an irregular contour and appearance, but there was no fixation to the pelvic side wall. Biopsies remained atypical but without evidence of invasive cancer. The patient remained reluctant to consider major operative treatment, which was repeatedly advised. A rectal biopsy performed at another hospital in April, 1974, was reported to show metastatic tumor.

Patient 3. M. U., a 65-year-old gravida 1, para 1 woman, had a supracervical hysterectomy in 1937 for a fibroid uterus. In May, 1968, a Class III Pap smear was obtained, although the cervix appeared to be grossly normal. She underwent total cervicectomy. The surgical specimen revealed invasive epidermoid carcinoma extending to the margins of the specimen. In September and October, 1968, she underwent external supervoltage radiotherapy to a dose of 400 r. to the midplane of the pelvis and had 80 mg. of radium by Burnet application for 48 hours in the vaginal vault. Following radiotherapy, she developed cystitis, urethral stricture, and, a year later, a rectovaginal fistula, treated by diverting colostomy.

In October, 1970, she was referred for evaluation of vaginal dysplasia and Class IV Pap smears, although no biopsies were performed. The vesicovaginal fistula was managed by ileal conduit urinary diversion. Repeated examination revealed persistent abnormal Pap smears consistent with severe dysplasia. The patient died in November, 1973, with extensive metastatic tumor apparently originating from the cervix.

Patient 4. C. J., a 63-year-old gravida 4, para 2 woman, underwent total abdominal hysterectomy and bilateral salpingo-oophorectomy in April, 1970, for carcinoma in situ of the cervix. A year later, Pap smears and vaginal biopsies showed noninvasive squamous-cell carcinoma. Multiple cytologic and histologic diagnoses over the ensuing 2 years have ranged from severe atypical hyperplasia to intraepithelial carcinoma of the vaginal apex.

Patient 5. F. L., a 54-year-old gravida 7 woman, presented in 1967 with symptoms of pelvic inflammatory disease. During treatment for her inflammatory disease, the patient was found to have squamous-cell carcinoma of the cervix. She underwent exploratory laparotomy and a large pelvic abscess was drained without excision of the cancer. She was then treated with external-beam orthovoltage radiotherapy and intracavitary radium. Four years later, monthly Pap smears from May to December, 1972, showed progression from suspicious cytology to dysplasia and then to carcinoma in situ.

Over a period of 2 years, multiple vaginal cytologic interpretations ranged from moderate dysplasia to epidermoid carcinoma, and multiple biopsies revealed carcinoma in situ without evidence of invasion. In February, 1973, four wedge biopsies were obtained from the cervix and vagina under general anesthesia and each showed carcinoma in situ. The patient was advised that excision of the vagina and cervix in such an extensively irradiated pelvis might entail the possibility of total pelvic exenteration. She declined this operation. Presently she is being followed for any evidence of invasion of her intraepithelial carcinoma.

Patient 6. E. H., a 52-year-old gravida 7, para 6, abortus 1 woman, in June, 1970, had a total abdominal hysterectomy and bilateral salpingo-oophorectomy after a cervical cone biopsy revealed in situ and invasive carcinoma of the cervix. Her cancer was found to be limited to the cervix and she had no tumor found in the lymph nodes removed with the surgical specimen. Two years later, a vaginal Pap smear was interpreted as carcinoma in situ; biopsy was done which showed only inflammation of the vaginal cuff without invasive tumor. Multiple biopsies over the ensuing years have shown chronic inflammation, focal ulceration, and focal carcinoma in situ in areas of epithelial atypia in the vaginal cuff.

Patient 7. G. S., a 32-year-old gravida 5, para 5 woman, was treated in 1950 with 6,000 mg.-hours of radium implantation and external-beam orthovoltage irradiation for a clinical Stage I epidermoid carcinoma

Table I. Summary of clinical information on 10 patients presenting with vaginal carcinoma in situ at intervals following treatment for cervical carcinoma

Patient	*Age at diagnosis of vaginal lesion*	*Date of diagnosis of carcinoma of cervix*	*Therapy for carcinoma of cervix*	*Date of diagnosis of vaginal lesion*	*Follow-up status of vaginal lesion*
1. L. S.	55	Oct. 1968	Total hysterectomy	March 1971	Persistent carcinoma in situ
2. J. W.	46	Nov. 1968	Radical hysterectomy, external radiation	March 1970	Invasive carcinoma
3. M. U.	65	May 1968	Cervicectomy, internal and external radiation	Oct. 1970	Persistent carcinoma in situ; died Nov. 1973
4. C. J.	63	Feb. 1970	Total hysterectomy	Feb. 1971	Persistent carcinoma in situ
5. F. L.	54	April 1967	Internal and external radiation	May 1972	Persistent carcinoma in situ
6. E. H.	52	Aug. 1970	Total hysterectomy	April 1972	Persistent carcinoma in situ
7. G. S.	55	Nov. 1950	Internal and external radiation	Oct. 1966	Persistent carcinoma in situ
8. P. E.	58	Feb. 1969	Total hysterectomy, external radiation	Jan. 1972	Developed invasive metastatic carcinoma of vulva; persistent carcinoma in situ of vagina (died 7 days after vulvectomy)
9. H. G.	38	Oct. 1966	Internal and external radiation	June 1972	Invasive carcinoma
10. A. B.	49	Oct. 1948	Internal and external radiation, radical hysterectomy	June 1972	Invasive carcinoma

of the cervix. In 1955 she had "suspicious" Pap smears but vaginal and cervical biopsies were negative. In 1966 a cytologic smear was suspicious but biopsies of the vaginal apex were normal. A year later, because of persistent Class IV Pap smears, she underwent total abdominal hysterectomy, bilateral salpingo-oophorectomy, and excision of the upper third of the vaginal apex. The surgical specimen showed no evidence of malignancy.

The patient was followed at 6 month intervals with normal cytologic patterns seen on Pap smear. Again, 4 years later, vaginal Pap smears were interpreted as Class IIB and continued in 1972 to be interpreted as Class II and Class III. In April, 1973, vaginal biopsies showed squamous-cell carcinoma in situ. Over the ensuing months, cytologic interpretations were consistent with carcinoma in situ and multiple vaginal biopsies showed marked or severe epithelial dysplasia.

Patient 8. P. E., a 59-year-old gravida 1, para 1 woman, was being followed at regular intervals after resection of an epidermoid carcinoma of the tongue in 1966. A routine Pap smear in 1969 showed Class IV changes and a cone biopsy showed carcinoma in situ. She underwent total abdominal hysterectomy and bilateral salpingo-oophorectomy. The surgical specimen showed invasive carcinoma of the cervix and she was treated by 5,800 r. tumor dose to her anterior pelvis and 6,000 r. to her posterior pelvis.

She remained asymptomatic and all Pap smears in the subsequent 2 years were normal. In 1972, routine Pap smear was interpreted as marked atypia and a vaginal biopsy revealed carcinoma in situ without evidence of invasion. A small mass was also noted on the right labium, which gradually enlarged.

A biopsy of the vulvar lesion revealed invasive squamous-cell carcinoma. She underwent vulvectomy, groin dissections, and anterior exenteration. Metastatic squamous-cell carcinoma was found in five of 24 pelvic lymph nodes, although no residual carcinoma was found in either vagina or vulva. Five days after her anterior exenteration she began massive bleeding from the upper gastrointestinal tract and died 2 days later. The histologic patterns of the squamous-cell carcinoma in the vulva resembled the lesions in the cervix and in the vagina.

Patient 9. H. G., a 38-year-old woman, had Stage Ib carcinoma of the cervix in 1966 during her fourth pregnancy. Pregnancy was interrupted by hysterotomy and she was treated by internal and external radiotherapy, delivering 10,000 and 4,500 r., respectively. Seven years later, in 1973, she experienced massive vaginal bleeding and biopsy of the anterior vaginal wall showed invasive squamous-cell carcinoma which was confirmed by repeated cytologic examinations.

Patient 10. A. B., a 27-year-old nulliparous woman, underwent internal and external radiotherapy with a 6,000 r. tumor dose followed by radical hysterectomy for squamous-cell carcinoma of the cervix. At age 52, in 1971, she noted vaginal spotting. Vaginal cytology and biopsy at another hospital revealed carcinoma in situ with questionable areas of invasion. On referral 3 months later, a friable vaginal nodule was biopsied which revealed invasive squamous-cell carcinoma. She underwent total pelvic exenteration. The surgical specimen showed invasive squamous-cell carcinoma of the remaining vagina extending into the paravaginal tissues without involvement of pelvic lymph nodes.

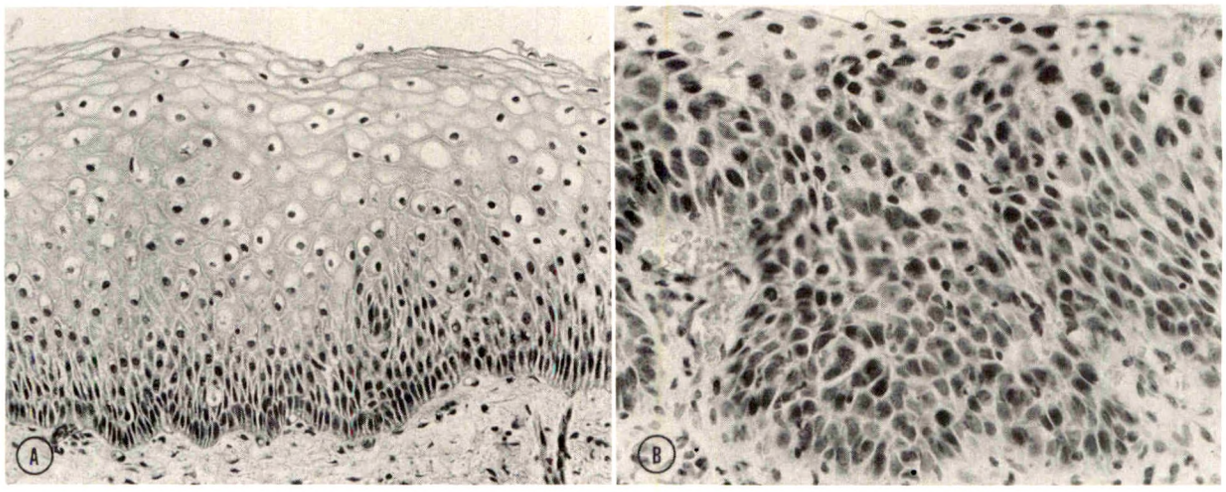


Fig. 1. Focal in situ carcinoma in biopsy specimen from Patient 4. *A*, Normal vaginal mucosa. (Hematoxylin and eosin stain; × 250 [S71-2206].) *B*, Carcinoma in situ of vagina. (Hematoxylin and eosin stain; X 350 [S71-2206].)

Summary of histologic findings

The histologic findings in these patients are summarized (Table I) because of the similarity of their dysplastic and carcinoma in situ features. The in situ vaginal lesions were focal in distribution in the vaginal mucosa (Fig. 1). Multiple biopsies from different areas of the vagina often revealed coexisting normal epithelium, mild atypia, dysplasia, and/or carcinoma in situ. In some sections junctions between dysplasia and in situ carcinoma were clearly evident. All histologic gradations, from mild atypia to unequivocal in situ carcinoma, were seen (Figs. 2 and 3). Malignant change was reflected by progressive cellular pleomorphism, disarray, and nuclear hyperchromatism.

The epithelium was only slightly hyperplastic, although rete ridges were lengthened and widened in some patients. Except for the cases of invasive carcinoma, the basement membrane under the epithelium remained intact. A mild chronic inflammatory reaction was occasionally present in the underlying stroma.

Atypical mitotic figures were numerous in all levels of the epithelium (Fig. 4). Surface keratinization was not evident, although an occasional dyskeratotic cell was found. The spectrum of histologic lesions was regarded as similar in all of our patient material. Morphologically, these lesions could not be differentiated from similar in situ lesions of the uterine cervix.

Comment

Certain characteristics which appears to be common in these cases of neoplastic vaginal change following carcinoma of the cervix do not appear to be shared by primary vaginal carcinoma. Unlike primary vaginal carcinoma, invasion is not an early feature and rarely is found on presentation. Whereas primary vaginal carcinoma often presents as vaginal bleeding or discharge, only one of these patients presented with bleeding or other evidence of invasion; the remaining nine were diagnosed because of abnormal vaginal smears. Histologically these in situ vaginal lesions did not resemble the leukoplakias, papillary lesions, or nodular ulcers characteristic of primary epidermoid carcinoma of the vagina.

The epithelial changes seen in the vagina following treatment for carcinoma of the cervix showed no regression in any of the patients we have examined. Unlike some dysplastic changes of the vulva which reportedly can revert toward a more nearly normal epithelium,[4] the vaginal atypia in each of these cases did not regress in any site where it had once started. Moreover, there appeared to be progression over a prolonged period to frank invasion in at least three of our cases. Progression from vaginal epithelial atypia to invasive carcinoma has been previously reported.[11]

Another common feature among the neoplastic vaginal epithelial changes seen in our series of patients that is not representative of primary vaginal carcinoma is the discrete focal distribution of these vaginal in situ carcinomas. Biopsies of the patients presented were carefully recorded as to the site from which the specimen was taken; frank carcinoma in situ as well as severe dysplastic changes were present in the vagina adjacent to areas of normal vaginal mucosa. Primary vaginal carcinoma, which represents about 1 per cent of gynecologic neoplasms, often arises as a malignant transformation within a wider tract of epithelium. The lesions we have seen in the vagina following treatment for cervical cancer have been focal in all instances.

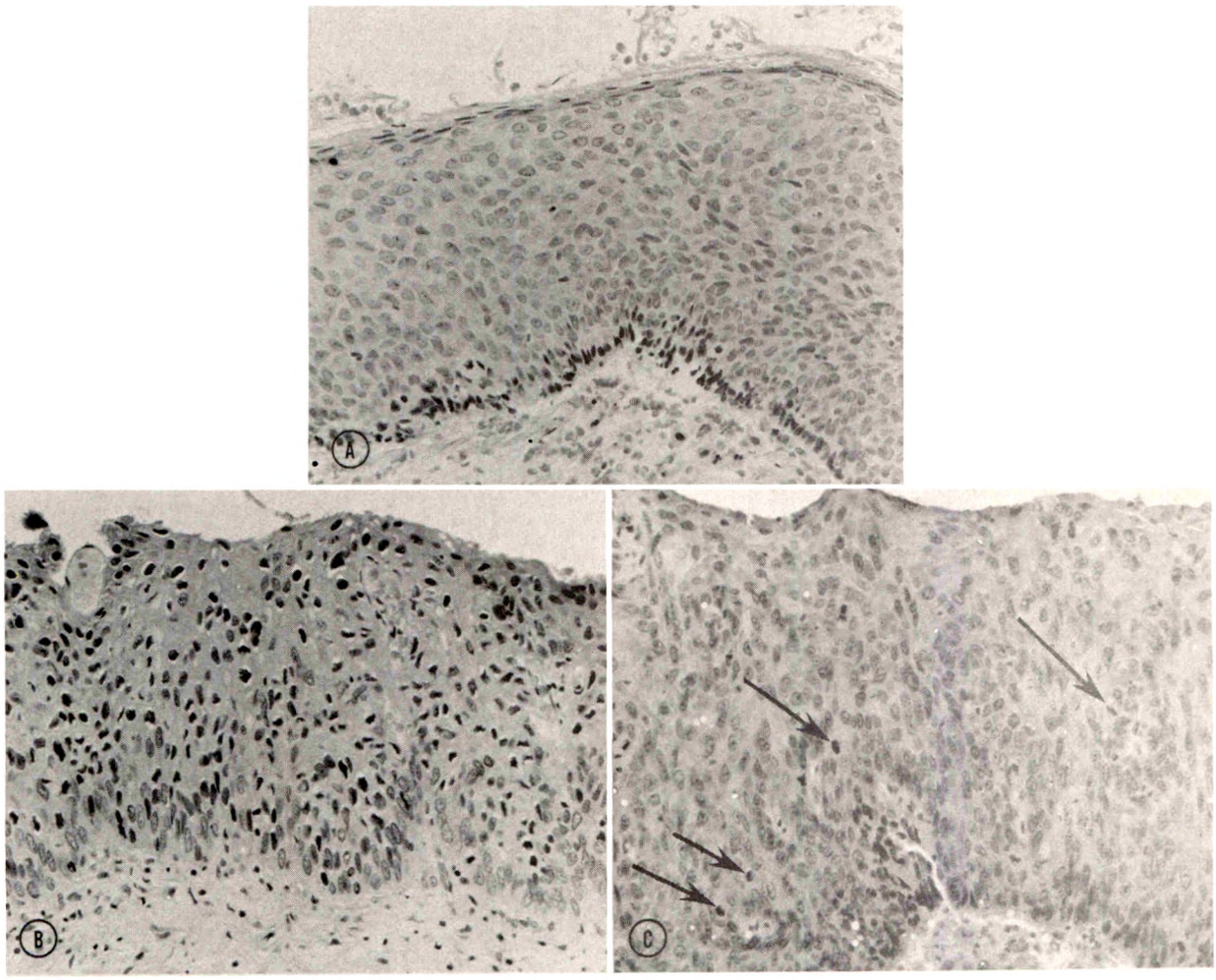


Fig. 2. Variation in histologic patterns of epithelial lesions in vagina of Patient 1. *A*, Minimally atypical vaginal epithelium with flattened cells along surface. (Hematoxylin and eosin stain; X 250 [S71-433].) *B*, More progressive area of atypism than in *A*. Nuclear hyperchromatism and pleomorphism are evident. This lesion qualifies as dysplasia. (Hematoxylin and eosin stain; X 250 [S71-985].) *C*, Another pattern of epithelial dysplasia. Arrows indicate numerous mitotic figures. (Hematoxylin and eosin stain; X 250 [S72-294].)

Radiation is known to cause atypical vaginal changes.[7] That radiotherapy cannot be implicated as the sole cause of these vaginal neoplastic changes is supported by two observations. First, three of our patients received no radiotherapy. In all our patients, the lesions were similar in clinical and pathologic characteristics whether or not the patient had received radiotherapy. Second, the epithelial changes were focal in distribution and not generalized to the entire area of the radiation field.

The carcinomas of the vagina seen in this series of patients following treatment for carcinoma of the cervix might be indicative of "field changes" in the ectogenital tract.[8] As such, many of the principles learned from long experience with carcinoma of the cervix in many clinical centers could perhaps apply to this vaginal carcinoma that follows cancer of the cervix. Our data indicate that in situ vaginal carcinoma precedes invasive vaginal carcinoma in much the same way and with a delay comparable to that reported for carcinoma of the cervix. Indeed, our study supports the suggestion that the natural history of cervical carcinoma and the vaginal carcinoma which follows cervical carcinoma are probably similar.[11]

After the initial detection of vaginal carcinoma during or following treatment for carcinoma of the cervix, it would appear that radical surgery encompassing the entire ectogenital tract might not be indicated at the time the lesion is first detected. Of 10 patients in our series followed for a mean period of 4 years, only

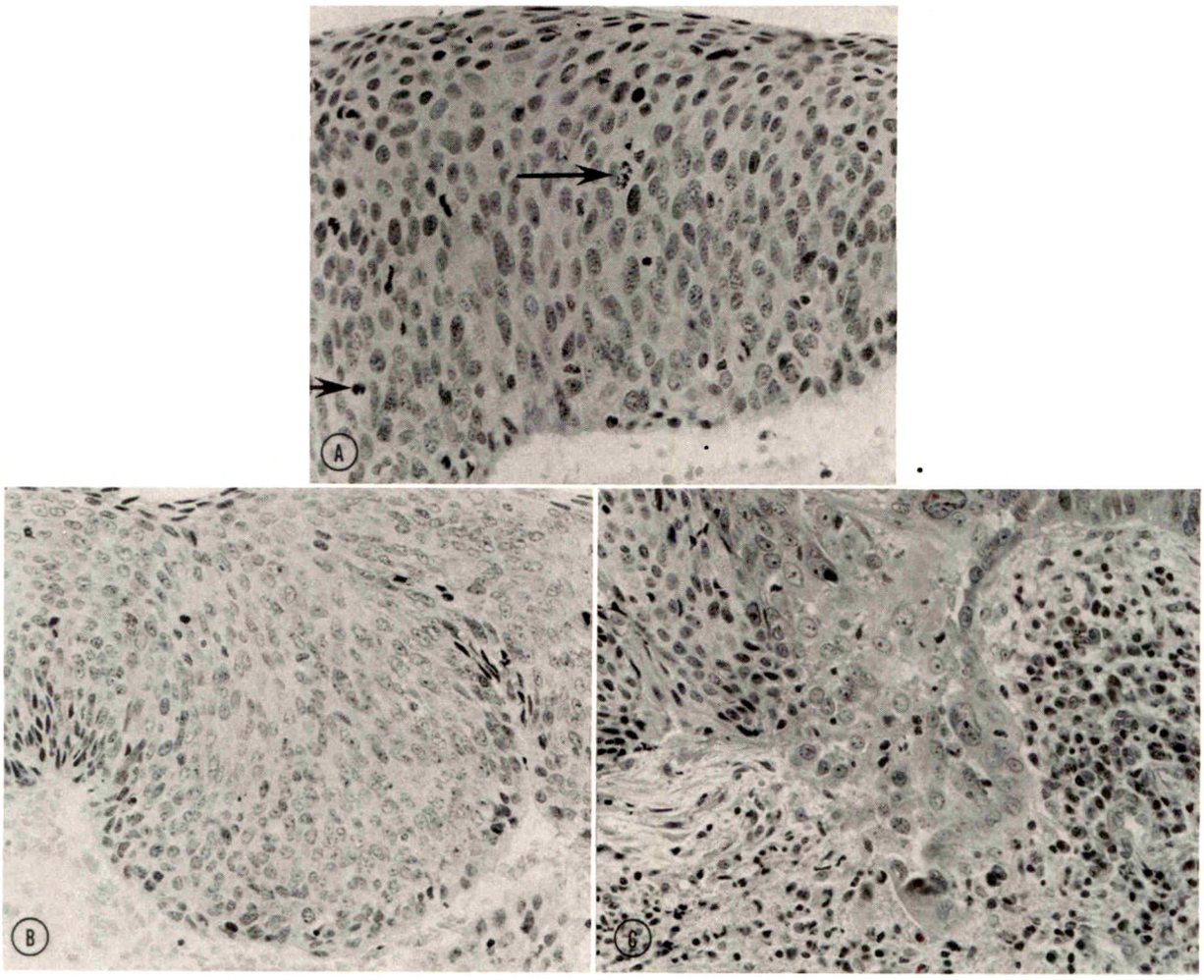


Fig. 3. Vaginal epithelial atypism and subsequent microinvasion in Patient 2. *A*, Moderately atypical epithelium with many mitotic figures (arrows). (Hematoxylin and eosin stain; X 340 [S71-1141].) *B*, Another section from the same tissue material as in *A* showing moderate dysplasia of the vaginal mucosa. (Hematoxylin and eosin stain; X 380 [S71-1141].) *C*, Focus of microinvasion. Tumor cells are extending into the stroma from an area of overlying carcinoma in situ (not shown on this photomicrograph). (Hematoxylin and eosin stain; X 320 [S71-2528].)

three have developed clinical and pathologic evidence of invasion. Careful follow-up and close evaluation of these patients has not resulted in a high morbidity or mortality rate to date. In each of these patients, a waiting period and re-biopsy has been done to check on the progression of this disease. Careful follow-up, however, is of crucial importance so that these patients might be given the benefit of treatment in the event that their carcinoma in situ progresses. Depending on the extent of the vaginal carcinoma in situ, local excision, vaginectomy, or exenterative therapy might be required in treatment.

A technique that might be applicable in the careful follow-up of these patients is that of colposcopy which has been used for close observation of in situ lesions of the cervix.[12] We have been following our patients by clinical examination, cytologic study, and repeated biopsies. In our experience biopsy is required because of our inability to distinguish areas of carcinoma in situ in fields of dysplastic epithelium by clinical examination.

Previous reports in the literature have noted an association of vaginal changes and surgical treatment or radiotherapy for carcinoma of the uterine cervix. Rutledge[11] reported 23 patients who developed in situ vaginal lesions following radiotherapy for carcinoma of the cervix. Histologically, it could not be determined whether the vaginal changes in these patients were malignant. Hummer and associates[6] reported that in 62 patients with carcinoma in situ of the vagina, 31 had

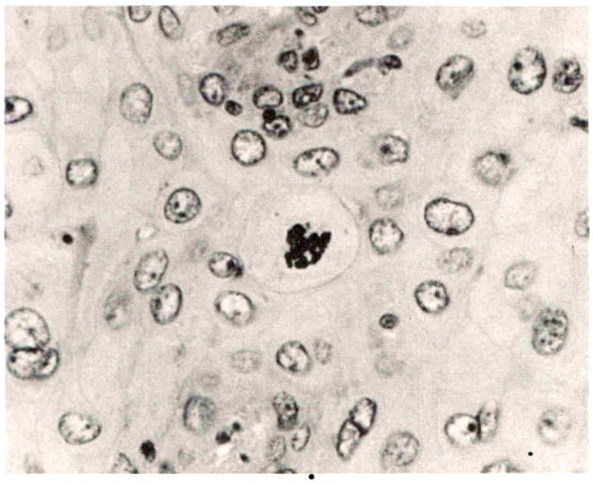


Fig. 4. Atypical mitotic figures with irregularly clumped chromatin in area of epithelial dysplasia (Patient 4). Hematoxylin and eosin stain; X 630 [S72-1204].)

carcinoma of the cervix or vulva simultaneous with the vaginal tumor and 24 had carcinoma of the cervix or vulva prior to the vaginal tumor. Other authors[1, 2, 5, 9, 13] have reported vaginal tumors associated with cervical cancer. Indeed this association has led Franklin and Rutledge[3] to postulate a common pathogenesis for epithelial tumors of the vulva, cervix, and/or vagina.

Although recent data suggest that carcinoma of the cervix is associated with latent herpes simplex type II, there are no studies to indicate that carcinoma of the vagina is associated with this virus infection. However, if the virus is involved in the etiopathogenesis of vaginal carcinoma following cervical carcinoma, we might assume that a wide area of ectogenital epithelium is exposed to the herpes simplex virus. Carcinoma of the vulva and vaginal carcinoma in situ were seen in one of our patients. Interestingly, squamous-cell carcinoma developed in the transplanted epithelium in the neovagina in one patient seen in our clinic who had a reconstruction following total pelvic exenteration as treatment for epidermoid carcinoma of the cervix.[10]

From this study we conclude that in situ changes in the vaginal mucosa associated with carcinoma of the cervix represent a field change in the ectogenital epithelium. This field change might predispose to separate primary carcinomas of the cervix and of the vagina arising at different points in time. Moreover, this field change is focal and exhibits a histologic continuum from mild epithelial atypia to carcinoma in situ and, in some patients, to invasive squamous-cell carcinoma. Our data indicate that in this disease dysplasia or carcinoma in situ of the vaginal mucosa can persist for many years in some patients without progressing to invasive carcinoma. These observations seem to indicate that the biologic behavior of carcinoma in situ of the vagina following carcinoma of the cervix resembles that of carcinoma in situ of the cervix.

Clinically, our results suggest that in some patients a conservative therapeutic approach might be indicated for vaginal in situ carcinoma developing after carcinoma of the cervix, unless the in situ lesions show evidence of progression. These findings also emphasize the need for unremitting long-term follow-up on patients with treated cervical cancer. Carcinoma of the vagina associated with cervical cancer should be considered a separate disease entity from primary invasive carcinoma of the vagina. A study of in situ vagina cancer might reveal clues to the pathogenesis and etiology of cervical cancer.

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In vitro ovulation from adult hamster ovary

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The purpose of the study was to achieve in vitro ovulation in order to acquire corpora lutea in vitro at "time zero" of their formation. Such in vitro corpora lutea would later be the subject of studies on maintenance and lysis of corpora lutea in vitro. Ovaries from adult hamsters, explanted into chemically defined medium between 23:00 hours on the day of proestrus and 01:00 hours on the day of estrus, were examined in the culture medium at 10:00 hours on the day of estrus, i.e., after 9 to 12 hours of incubation. Ovulated follicles (young corpora lutea) of differing stages of corpus luteum formation were observed in some of the explants, but not all. Almost all ovulated ova, surrounded by their cumuli oophori, remained adherent to the area of rupture when the incubation was ended. In vitro ovulation occurs in chemically defined medium without added hormones. It is possible to obtain corpora lutea in vitro at "time zero" of their formation.

IN THIS LABORATORY an ongoing interest in mechanisms of ovulation and in the maintenance and destruction of corpora lutea[1, 4] has been reinforced by the utilization of in vitro organ culture methodology.[2, 3]

To study the endocrine control of the maintenance of corpora lutea, the rescue of corpora lutea from incipient or inevitable luteolysis, and the control of luteolysis, a desirable model system would include knowledge of—or the ability to monitor—"time zero" of corpus luteum formation. Such a system could have advantages over those in which luteal transformation of cells may appear to have occurred in vitro, or those in which corpora lutea are obtained from an animal and arbitrarily dated or not dated in subsequent experiments.

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Materials and methods

Adult, cycling, golden hamsters (60 to 100 grams, Con Olson) were housed at constant temperature (23° C.), fed Purina Lab Chow and water ad libitum, and maintained on a 14 hour light–10 hour dark schedule (lights on at 05:00, off at 19:00 C.S.T.). The hamsters were monitored for three consecutive 4 day cycles and then used in the experiments. The day of ovulation, characterized by a conspicuous vaginal discharge, was called day 1 of the cycle. The hamsters were killed by cervical dislocation at 23:00 and 24:00 hours on the day of proestrus, i.e., day 4 of their cycle, and at 01:00 hours on the day of estrus.

The ovaries were transferred aseptically into plastic Petri dishes (Lab-Tek Plastic Petri Dishes No. 4016), where removal of fat and stripping of the bursa and oviduct were performed aseptically in an Edgegard laminar flow hood (Model No. 4252). The ovaries were transferred to culture vessels (Bio Quest Organ Culture Dishes No. 3010) containing 1 ml. of modified culture medium 199.[3]

To preclude the possibility of any pH shifts, the medium was modified to contain 2.2 Gm. per liter of sodium bicarbonate and the gas within the culture chamber contained 5 per cent CO_2. The pH of the modified medium 199 was 7.46. The media contained an indicator (phenol red) which at the working pH was a pinkish red. No change in color was observed during or after the incubation periods.

In some culture vessels this culture medium con-

tained progesterone (Sigma), 4.8 μg per milliliter of medium. The pH of the medium with progesterone was 7.55. It should be added that progesterone is not an osmoactive compound and available data indicate that no permeability barrier exists at the cell membrane for progesterone,[5] so that progesterone readily equilibrates within cells.

The loaded culture vessels were placed in culture chambers[2] and immediately gassed (95 per cent O_2–5 per cent CO_2, premixed, Puritan Bennett Corporation) for 4 minutes at the rate of 10 L. per minute. The time interval from cervical dislocation to culture was less than 2 minutes. The contralateral ovary from each animal provided an internal, uncultured, and unovulated control for the ovary incubated in vitro. The control ovary was dissected in the same manner as the ovary that was incubated, then immediately fixed in Bouin's.

The culture chamber, containing up to six culture vessels, was housed in a Precision Thelco Incubator (Model 6) at 34° C. until 10:00 hours of the following day. Oxygen content of the culture chambers was measured[2] 1 hour prior to the termination of the experiment. At the end of the incubation, the culture vessels were examined under a dissecting microscope for the presence of fresh ova. Photomicrographs were taken. The incubated ovaries were fixed in Bouin's. The fixed ovaries, both cultured and uncultured, were dehydrated through a graded ethanol series, cleared, embedded in paraffin, serially sectioned at 6 μ, and stained with hematoxylin and eosin.

Results

The hamster ovary (Fig. 1, *A*), incubated in basic medium, with no exogenous hormonal support, did not ovulate in vitro in every case. Large antral follicles are easily discernable and the presence of an ovum within the follicle can be seen (Fig. 1, *B*). There is no visible damage due to the dissection or manipulation of the ovary, and no evidence of ovulation.

In vitro ovulations did, however, occur from such ovaries incubated in media without progesterone. When the ovaries were explanted at 23:00 hours and 24:00 hours of proestrus, and 01:00 of estrus, the frequency of ovulated ovaries was 1/6, 2/6, and 4/6 respectively, and there was only one ovum ovulated per ovary in each case. With progesterone in the medium (Fig. 1, *C, D*) the respective figures were 5/5, 6/6, and 3/3 approximating the normal in vivo complement, ensued.

There was no evidence of degeneration of the ovulated ova (Fig. 1, *E*). In such ova, the second meiotic spindle is visible under the membrane, parallel to the surface and perpendicular to the position of the first spindle. Corona radiata cells surround the ova in each case. The incubated ovary (Fig. 2, *A, C, E*) and the contralateral unincubated ovary from the same animal (Fig. 2, *B, D, F*) illustrate that there are no significant differences between the two. Both ovaries contained preovulatory ova (Fig. 2) undergoing meiotic division and maturation. The unovulated ova, whether from control (Fig. 2, *B, D, F*) or experimental (Fig. 2, *A, C, E*) ovaries, are within their cumuli oophori, detached from the membrana granulosa (Fig. 2, *A, B*) and free floating within the follicular fluid (Fig. 2, *C, D*).

The ruptured follicle or newly formed CL (Fig. 1, *C, D*) does not collapse at the time of ovum release and the ovulated ovum and granulosa cells adhere to the freshly ruptured areas of the follicular wall. The rupture in the follicle or newly formed CL initially appears to be closed by a single layer of cells (Fig. 3, *B*). The residual antrum persists in a somewhat collapsed state for a period of time and there is a proliferation of cells from the inner follicular wall of the presumptive CL into the residual cavity (Fig. 3, *A, D*).

In the postovulatory period, the markedly translucent area about the rupture point (Fig. 1, *C*) is indented (Fig. 3, *C*) due to a partial collapse of the follicle during early CL formation. The ruptured wall is then reinforced by several cell layers (Fig. 3, *C, E*), while the residual antrum is further compressed (Fig. 3, *E*).

Comment

Our experimental design was chosen so as to ensure that necessary follicular maturation changes preceding ovulation would be accomplished in vivo by the animal. Such ovaries, containing mature preovulatory follicles, were destined to ovulate in vivo. When explanted into the basic culture medium with no hormones added, in vitro ovulation did not occur as regularly as in the animal. The few ovaries that did ovulate in vitro released a fraction of the normal full complement of ova. When progesterone was added to the culture medium, however, the normal full complement of ova was ovulated in vitro from every incubated hamster ovary. In this light, another species and model is being tested in our laboratory. Ovaries obtained from PMS-stimulated, immature rats have been incubated with progesterone under the same conditions as the hamster ovaries; they also yielded a profuse number of in vitro ovulations (Baranczuk and Fainstat, unpublished observations).

Our physical manipulation of the ovary at explantation did not, in itself, cause in vitro ovulation. The ovaries were manipulated during our dissections, transferred, and incubated without any apparent

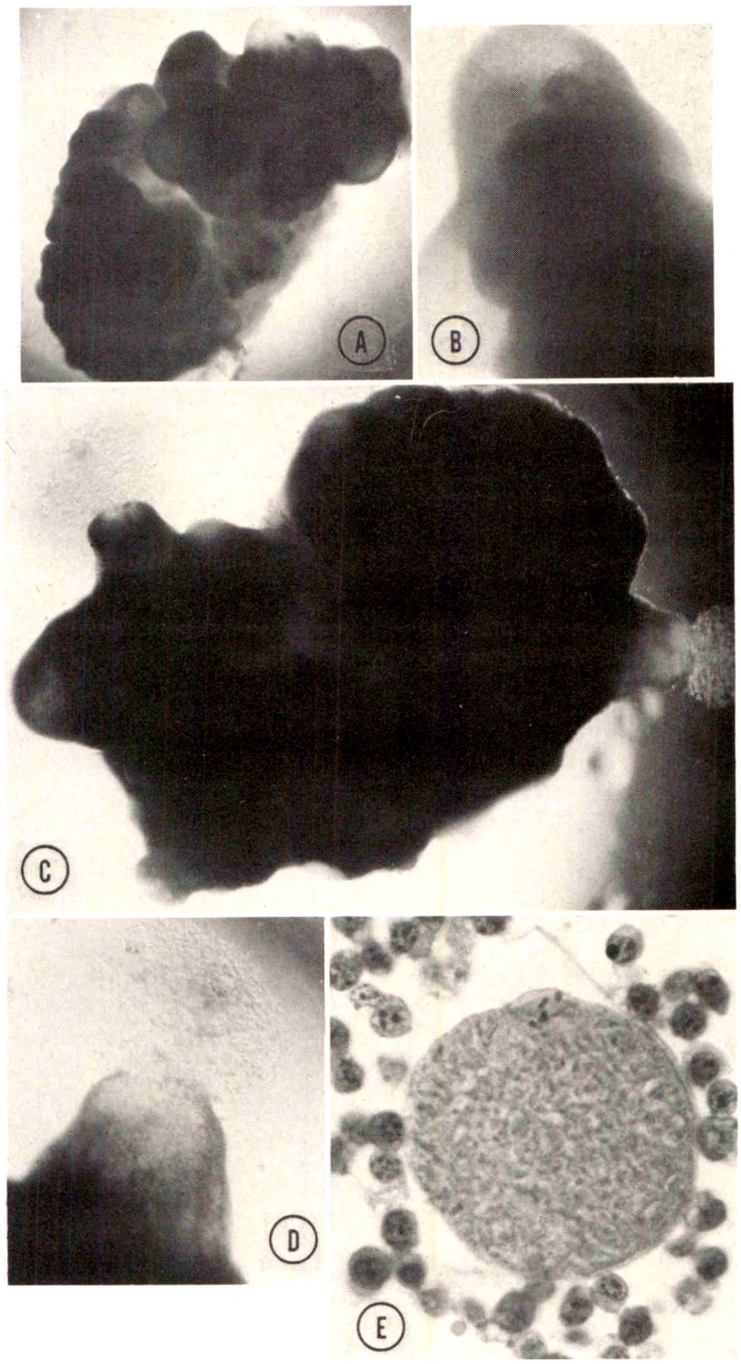


Fig. 1. *A,* Hamster ovary explanted at 23:30 hours on day of proestrus and incubated in medium without progesterone until 10:00 hours of next day. Note multiple, large, unovulated follicles. Manipulation and dissection during experimental procedure did not cause follicular rupture or ovulation. (×75.) *B,* Magnification of portion of ovary in Fig. 1, *A*. Ovum in normal location within unovulated follicle. There is no displacement of the ovum nor visible damage to the follicles due to experimental procedures. (×225.) *C,* Hamster ovary explanted at 23:15 hours on day of proestrus and incubated in medium containing progesterone, 4.8 μg per milliliter, until 10:00 hours of the next day. Multiple follicular ruptures are visible. (×75.) *D,* Magnification of portion of ovary in Fig. 1, *C*. A freshly escaped ovum, within its cumuli oophori, is loosely attached to the area of follicular rupture. (×200.) *E,* In vitro ovulated hamster ovum surrounded by corona radiata. Metaphase II chromosome configuration visible. (×750.)

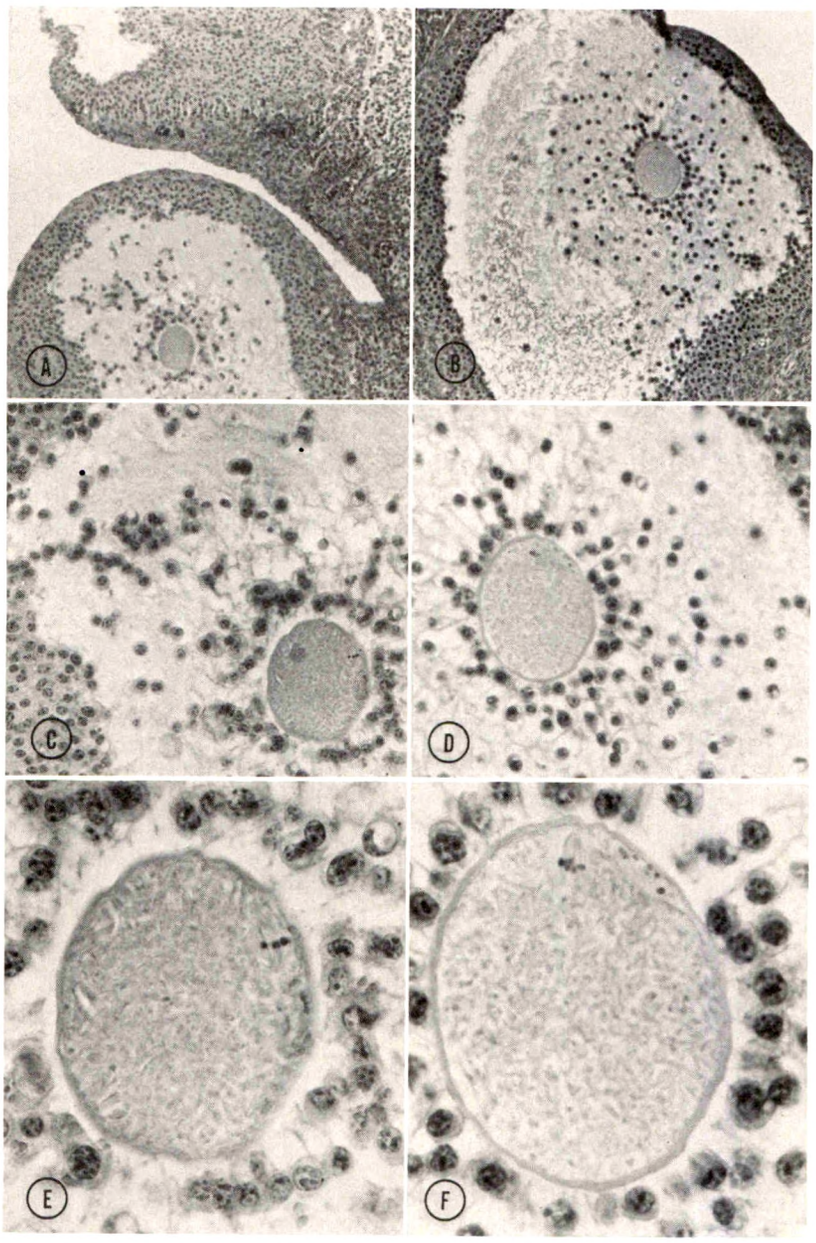


Fig. 2. *A, C, E,* In vitro ovulated and unovulated follicles in hamster ovary incubated with progesterone. *A,* Section of ovary incubated for 10 hours with progesterone shows unovulated follicle below and young CL above with collapsed antrum and rupture point sealed by single layer of peripheral cells. (×122.) *C,* Preovulatory ovum within cumulus oophorus, detached from membrana granulosa and free-floating within follicular liquor, is from follicle shown in *A,* above. (×310.) *E,* Higher magnification of ovum in *C,* above, shows second meiotic spindle parallel to egg membrane surface and perpendicular to position of first meiotic spindle, as shown in *F,* at right. (×900.) Meiosis continued throughout the incubations and the corona radiata continued to surround ova. *B, D, F,* Contralateral control ovary from same animal as experimental ovary in left column. This ovary was fixed when ovary at left was explanted in vitro. This unincubated ovary shows state of follicular components at beginning of 10 hours of incubation. *B,* No ovulations occurred in this ovary, but the states of preovulatory follicles in *B* and *A* are essentially identical. (×120.) *D,* Preovulatory follicle is similar to *C,* but corona in *C* may have undergone very early disaggregation of periovular cells. (×375.) *F,* Higher magnification of *D* shows ovum 10 hours younger than ovum in *E,* at left. Late anaphase of first meiotic division aligned perpendicular to egg membrane in contrast to *E,* showing metaphase II parallel to membrane. (×960.)

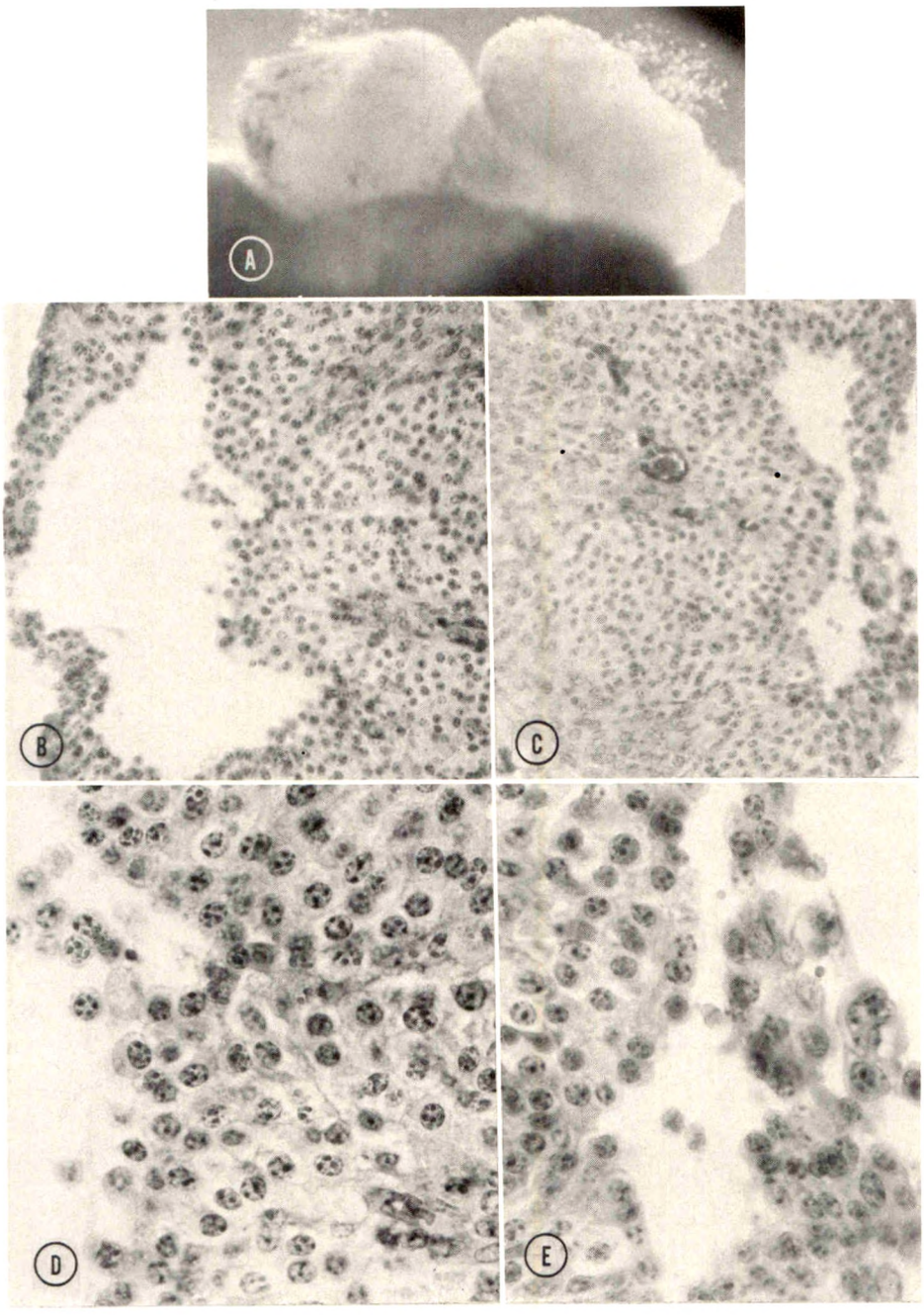


Fig. 3. *A*, Portion of hamster ovary explanted at 23:45 hours on day of proestrus and incubated in medium containing progesterone, 4.8 μg per milliliter, until 10:00 hours of next day. Two ova were teased away from ovarian surface. However, a portion of the cumulus containing residual granulosa cells remains adherent to both sites of follicular rupture. Exact time of ovulation not known. The partial collapse of the presumptive corpora lutea indicates that the ovulations probably occurred earlier than those in Fig. 1, *C* and *D*. (×225.) *B* and *D*, Higher magnifications of young CL in Fig. 2, *A*. Point of rupture at left is closed by a single layer of cells. Antral cavity is partially collapsed. There is an apparent early proliferation of cells from the base of the follicle wall into remaining follicular liquor. (*B*, ×300; *D*, ×960.) *C* and *E*, Sections from ovary treated as described in *A*, but demonstrating evidence of probably earlier in vitro ovulation and more advanced CL development, although treatments and timing were same as in *B* and *D*. The greater degree of follicle wall collapse, smaller amount of antral fluid, and increased thickness of follicle wall at site of stigma are representative of the appearance of the CL on the right in Fig. 3, *A*. (*C*, ×300; *E*, ×960.)

damage to the antral follicles protruding from the surface of the ovary (Fig. 1, *A*, *B*). There was neither breakdown of the follicular wall nor displacement of the ovum within the antrum. Neal and Baker[6] reported that in vitro ovulation in the mouse was a rare event. They suggested that it was possibly due to the mechanical damage caused during the preparation of the explants. It appears more likely that the rare ovulations in their in vitro experiments correspond to the rare ovulations seen in our studies in the absence of progesterone.

With progesterone in the chemically defined culture medium, 100 per cent of the explanted ovaries ovulated. Furthermore, multiple ovulations were monitored from each ovary (Fig. 1, *C*). Progesterone was an essential part of the ovulatory responses observed. Rondell[8] has postulated that the steroid synthetic processes, particularly the synthesis of progesterone, are essential intermediates in the normal in vivo ovulatory process. Indeed, it is now clearer than before that gonadotropic stimuli to the ovary set in motion a series of active metabolic processes which culminate in ovulation, and local progesterone action on the follicle is important in the ovulation mechanism.

Explanting and incubating do not appear to adversely affect the ova in our experiments. Our data do not confirm the belief that mammalian follicles are too large and too active metabolically for adequate respiratory exchange in culture.[8] The ovulated ova (Fig. 1, *D*, *E*) exhibited no evidence of degeneration. Meiosis continued throughout the incubations and the corona radiata cells continued to surround each postovulatory ovum. This was further apparent on examination of unovulated, incubated follicles and their unincubated contralateral controls. The status of the ova in both types of ovaries appeared identical to each other (Fig. 2) and to those described by Witschi[9] for vertebrates in general and by Norman and Greenwald[7] for hamsters in particular.

The ruptured follicle or presumptive CL also showed no signs of degeneration. Moreover, reconstruction and remodelling of the wounded follicular structure was evident. There was repair of the wound in the follicle wall (Fig. 3, *B*), a proliferation of cells from the walls into the residual antral cavity (Fig. 3, *D*, *E*), a compression of the cavity (Fig. 3, *A*, *B*, *C*), and reinforcement of the wounded wall by additional layers of cells (Fig. 3, *C*, *E*).

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Epidermoid cysts of the ovary: A report of five cases with histogenetic considerations and ultrastructural findings

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Five cases of epidermoid cysts of the ovary are added to the two reported in the literature. This rare lesion was uniformly an incidental finding in the study of hysterectomy specimens. Its main interest is in its histogenesis, which is suggested to involve metaplasia of the coelomic surface epithelium of the ovary. Ultrastructural evidence is one case strongly favors this possibility, thus adding another line of differentiation to those already established for the multipotent coelomic epithelium. However, some cases may be teratoid in origin, as has been suggested for the analogous lesion in the testis.



EPIDERMOID CYSTS of the ovary are defined as cystic structures lined solely by mature squamous epithelium and containing only keratinized debris. They represent an extremely rare condition in the ovary, with only two cases reported in the recent English literature.[1] In the testis, although they are also rare, they seem to be numerically significant, and a recent article from the Armed Forces Institute of Pathology reported a review of 69 cases.[2] To our knowledge, this is the first report on epidermoid cysts of the ovary in the American literature.

Material and methods

Four cases of ovarian epidermoid cysts were found among 1,650 consecutive ovarian neoplasms, an incidence of 0.24 per cent. An additional case was seen in more recent material. Tissue was fixed and stained routinely, and in four cases, serial sections of the lesion-containing blocks were obtained. Formalin-fixed tissue from Case No. 5 was repeatedly washed in S-collidine buffer, osmicated, and embedded in Epon-Araldite mix; ultrathin sections were stained with lead citrate and uranyl acetate and examined with a Philips 200 electron microscope.

The light microscopic criteria of selection included the following: (1) cyst contents composed exclusively of keratinized debris, with absence of hair and/or sebaceous secretion; (2) absence of other teratoid tissue components within the neoplasm, such as cutaneous adnexa, respiratory organ–like epithelium, etc.; (3) absence of endometrial, mucinous, or transitional-type epithelia; (4) absence of a granulomatous reaction within the wall which may have effaced other tissue components.

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Results

All cases were coincidental findings in total abdominal hysterectomy specimens (Table I). In two instances, the operations were performed because of multiple leiomyomas, in one instance, because of adenomyosis; in one, because of adenoacanthoma of the endometrium; and in one, because of metrorrhagia with no macroscopic or mircoscopic uterine lesion. The ovarian lesion was not clinically suspected in any case. All lesions were unilateral. The patients ranged from 29 to 64 years old, with a mean age of 44 years. Only the oldest patient was postmenopausal.

Gross findings. The cyst diameters ranged from 2 to 14 mm., with a mean of 7.4 mm. They were well delineated from the ovarian parenchyma, with smooth inner surfaces and lumina containing keratin debris (Fig. 1). Four lesions were unilocular and only one was

Table I. Epidermoid cysts of the ovary

Case No.	*Age (yr.)*	*Side*	*Size (mm.)*	*Location*	*Histologic features*	*Other findings*
1	43	Left	12	Subcortical	Granular layer, calcified keratin	Leiomyomas
2	53	Left	2	Subcortical	Absent granular layer	Adenomyosis
3	29	Right	3	Subcortical	Granular layer, calcified keratin, multilocular	—
4	33	Left	6	Hilar	Granular layer	Leiomyomas
5	64	Left	14	Subcortical	Granular layer	Endometrial adenoacanthoma



Fig. 1. Gross appearance of a small, unilocular, smooth-walled cyst containing keratin debris (Case No. 5, Colorado University Medical Center, Pathology Department, No. 74S-6567).

multilocular. In four instances, the cysts were situated cortically or paracortically, and one was found in the ovarian hilus. In all cases, the ovaries had an unremarkable external appearance, and the lesions were found only after the ovaries were sectioned.

Histologic findings. The cysts were delineated from the ovarian parenchyma by a band of fibrous tissue in which no inflammatory reaction was present (Fig. 2). No evidence of adjacent scar tissue was present at any level. The lining consisted of a thin squamous epithelium in which intercellular bridges were easily recognized (Fig. 2). In all but one cyst a prominent granular cell layer was present (Fig. 3). No atypism was noted at any level. In the multicystic lesion, all cavities were lined by the same kind of epithelium. The cyst contents were concentrically laminated keratinized material, calcified in two instances.

No in-foldings of the coelomic surface ovarian lining were noted, except in Case No. 3 in which a minute germinal inclusion cyst was present and contained a psammoma body. This cyst was not continuous with the epidermoid cyst, and there was no evidence of continuity between the surface epithelium and the epidermoid cyst in any case. In the hilar cyst, serial sections did not show any communication with hilar mesonephric remnants, which were not identified in sections from this ovary. The cyst was not surrounded by smooth muscle, as often found around rete structures. The histology of the ovarian parenchyma in all five cases was compatible with the age of the patient, and no special features were noted. No inflammatory foci were present at any level in any case.

Electron microscopic findings. In Case No. 5 the cyst lining with three to four cell layers rested on a thin basal lamina surrounded by abundant collagen fibers (Fig. 4). The lining cells were uniform in size and polygonal in shape. Complex interdigitated plasma membranes were present with well-formed desmosomal attachments, more prominent toward the luminal border, where terminal junctions were also noted (Fig. 5). The luminal border demonstrated numerous microvilli. The cytoplasm contained large numbers of aggregated tonofilaments, converging on desmosomal junctional complexes (Fig. 4). Pinocytotic vesicles were present along the paraluminal cell margins, mitochondria were scanty, and profiles of both granular and agranular endoplasmic reticulum were few in number but widely dilated. Moderate numbers of non–membrane-bound lipid droplets were present, as were large autophagic vacuoles. Nuclei showed relatively uniform chromatin, with few peripherally or centrally situated nucleoli. The nuclear membrane showed frequent in-foldings, with numerous groovelike cytoplasmic invaginations (Figs. 4 and 5).

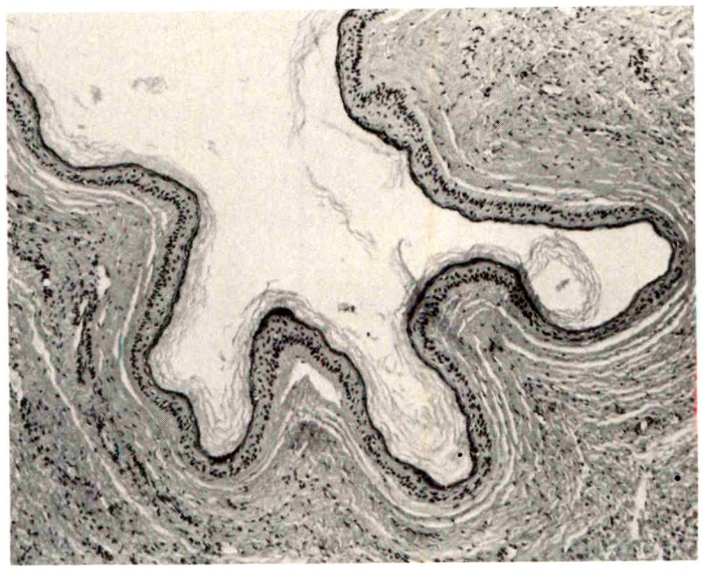


Fig. 2. Low-power photomicrograph showing pure squamous epithelium-lined cyst containing keratin debris and surrounded by fibrous tissue. (Hematoxylin and eosin. Original magnification ×60.)

Comment

These cysts appear to be of no clinical significance because of their small size and the absence of atypia of their linings, although a recent report[3] illustrates a primary pure squamous cell carcinoma of the ovary which may perhaps represent a malignant counterpart. Their small size is due to the sole presence of squamous epithelium with slow and scanty shedding of keratin and absence of secretion of any fluids which may increase their size and thus make them clinically evident. No scar tissue was present at any level to suggest a mature remnant of a previous locally healed teratoid malignancy, as in cases reported in the testis.[4]

The main question which this lesion raises is that of its histogenesis. In the testis, two likely origins have been considered. The most probable is a one-sided development of a teratoma,[2] as illustrated by carcinoid and retinal anlage tumors in both the testis and the ovary. Another possibility is that of metaplasia of the rete testis structures, as was claimed in one instance.[5] The single case report claiming an origin from seminiferous tubules[6] is not well documented.

To these possibilities we may add another in the ovary: the coelomic surface epithelium, which is known to possess the histogenetic potential for differentiation into tubal, endometrial, endocervical, and transitional-type epithelia. Within these differentiations, squamous metaplasia may occur in the endometrial, endocervical, or transitional epithelium. Isolated squamous metaplasia of the peritoneum has also been documented in the literature,[7] and we have seen this pattern in the surface lining epithelium in the parahilar region in a case which was not Müllerian in origin. There is no reason to doubt pure squamous metaplasia as one of

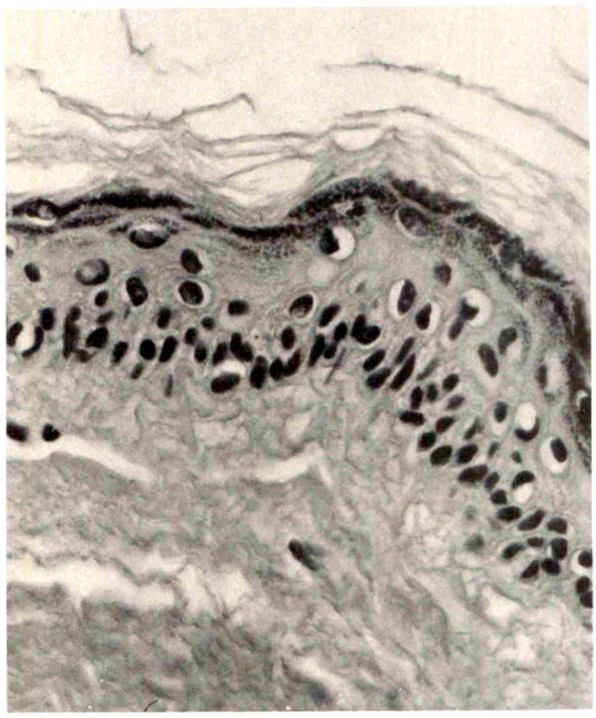


Fig. 3. Detail of lining showing stratified squamous epithelium with a prominent granular layer and without atypia. (Hematoxylin and eosin. Original magnification ×300.)

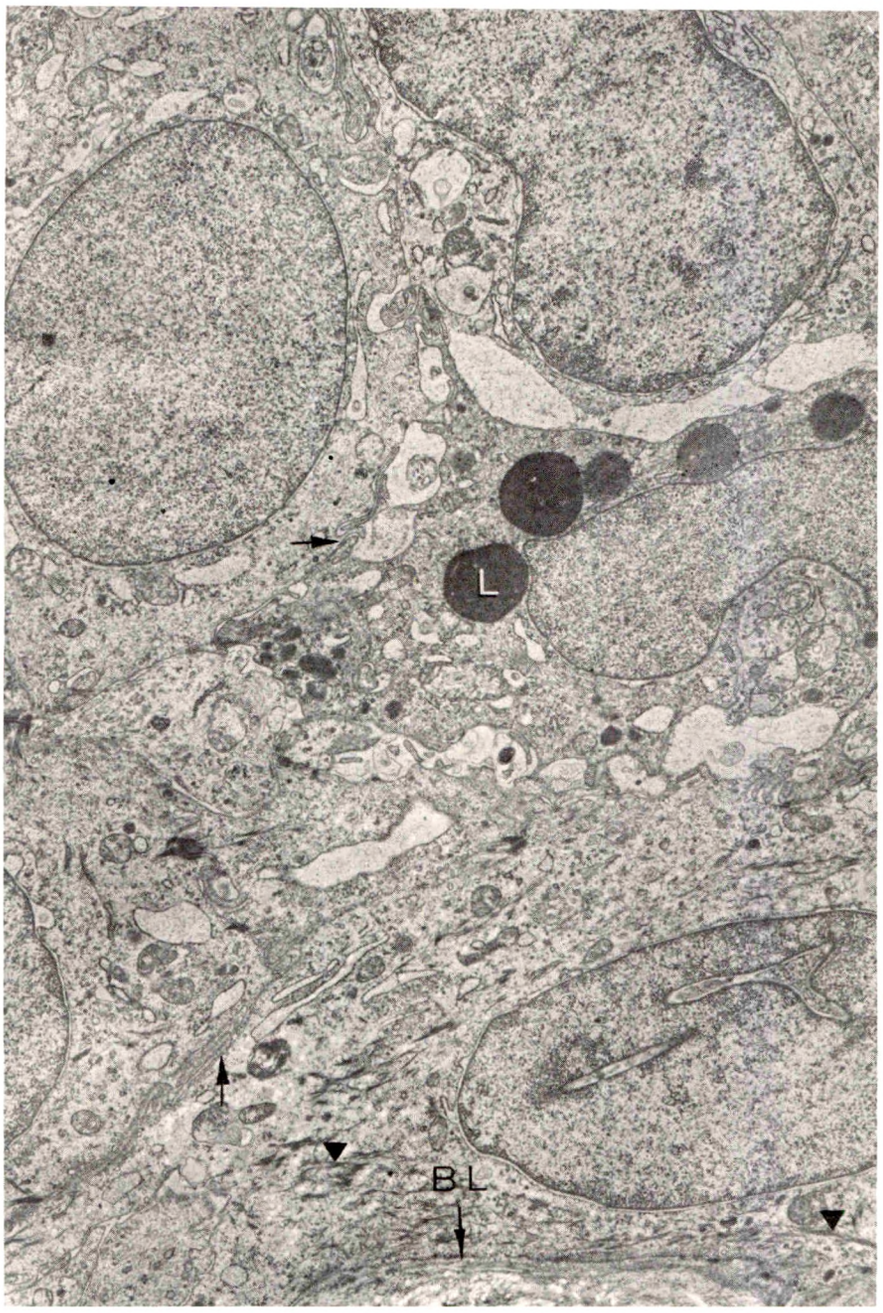


Fig. 4. Electron micrograph (Case No. 5) showing epithelial cells separated from collagen fibers by a basal lamina *(BL)*. The cell at the bottom of the figure contains numerous tonofilaments (arrowheads). Lipid droplets *(L)* are prominent, as are interdigitating cytoplasmic membranes (arrows). The nucleus at lower right shows invaginations of cytoplasm. (×10,080.)

the potentialities of the Müllerian derivatives of the coelomic epithelium. The presence of a well established granular layer in our cases, otherwise not found in normal squamous epithelium of presumed Müllerian origin, does not rule out such an origin, since these epithelia and their neoplastic derivatives can under adequate stimuli develop keratinization, as in prolapse of the cervix, endometrial adenocanthoma,[8] ichthyosis uteri, and ovarian mucinous[9] and Brenner tumors.[10]

Ultrastructural findings in Case No. 5 support the notion of a metaplasia of coelomic-derived epithelium.



Fig. 5. Electron micrograph of luminal surface of cyst (Case No. 5), showing numerous blunt microvilli and desmosomal junctions with tonofilaments (arrows). Other thin filaments are prominent in the cytoplasm. Dilated intercellular spaces contain cytoplasmic processes *(P)*. A few profiles of dilated granular endoplasmic reticulum *(E)* are seen. The nucleus is prominently indented. (×12,340.)

The cyst contents were typical laminated keratin (Fig. 1), and optical microscopy showed the presence of a granular cell layer in most areas. However, cells lining the cyst were characterized ultrastructurally by surface microvilli, complex interdigitating plasma membranes with prominent desmosomal junctions, numerous intracytoplasmic lipid droplets and autophagic vacuoles, frequent in-foldings of the nuclear membrane with numerous nuclear grooves, and relative paucity of mitochondria and endoplasmic reticulum. These findings are characteristic of Walthard cells nests and of Brenner tumors, both of which are derived from the

coelomic epithelium.[11–13] An unusual finding in this case was the presence of numerous tonofilaments, the aggregation of which represented the ultrastructural manifestation of the squamous metaplasia noted at the optical microscopic level. Since the ovarian cyst in this case coexisted with an adenoacanthoma of the endometrium, perhaps the stimulus, hormonal or otherwise, to squamous metaplasia affected both tissues.

Case No. 4 suggests possible metaplasia of the rete ovarii, as has been considered in the homologous lesion in the testis.[5] Arguments have been advanced for both a coelomic and a mesonephric derivation of the rete ovarii,[14] so this case may or may not also favor an ultimate coelomic origin for the epidermoid cyst. Similarly, it has also been considered that coelomic-derived neoplasms such as the Brenner tumor may have originated in the rete when they are hilar in location.[12]

Despite these suggestions, we have no way to discard a possible teratoid origin for these lesions in some cases, and arguments favorable to this possibility may be equally solid. It may be pertinent to mention that we have seen a sixth ovarian lesion identical to the five herein reported except for its larger size, intraluminal hairs, and a mild granulomatous reaction in the stroma. Since this case was excluded from the present series by the criteria defined above, it is difficult to ascertain its possible relationship to the other five cases.

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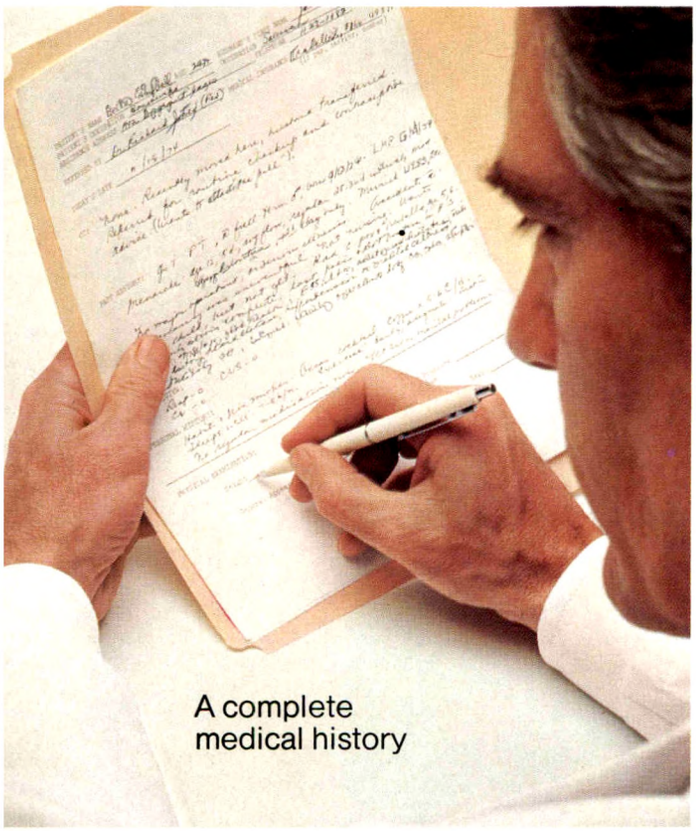
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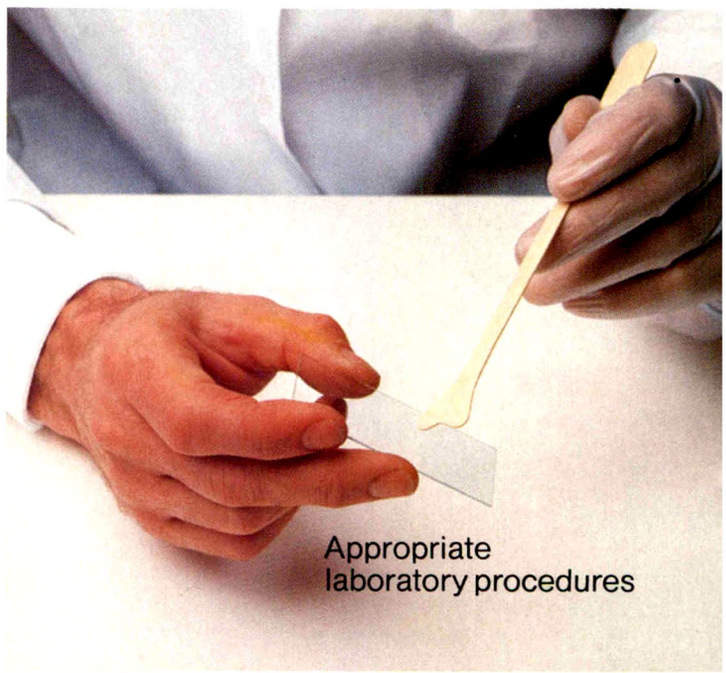
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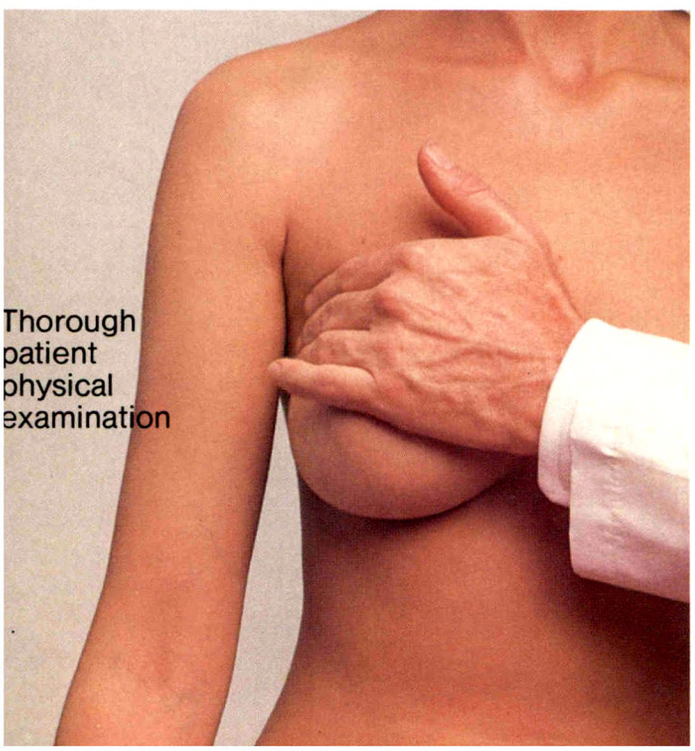
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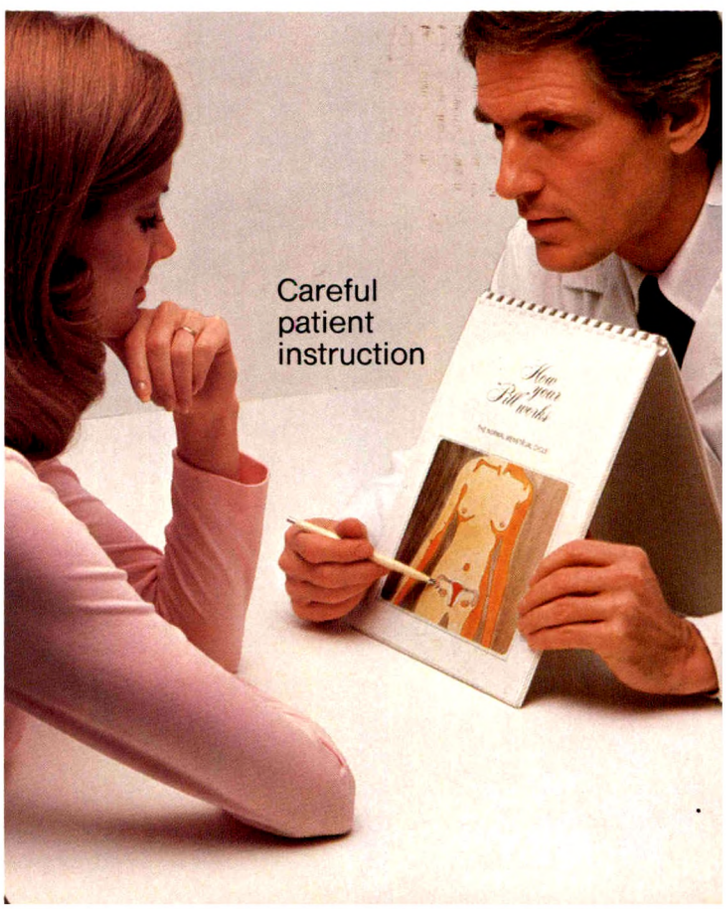
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ethinyl estradiol (17 alpha-ethinyl-1,3,5 (10)-estratriene-3, 17 beta-diol) . . . . . 20 mcg
Each brown tablet contains
75 mg ferrous fumarate, USP

BRIEF SUMMARY OF
PRESCRIBING INFORMATION

SPECIAL NOTE

Oral contraceptives have been marketed in the United States since 1960. Reported pregnancy rates vary from product to product. The effectiveness of Loestrin 1/20 is somewhat lower than that of the higher-dose combination products. Both provide almost completely effective contraception.

The dropout rate for medical reasons, as observed in the clinical trials that were conducted with Loestrin 1/20, appears to be somewhat higher than observed with higher-dose combination products. This higher dropout rate was observed to be due particularly to irregular bleeding.

An increased risk of thromboembolic disease associated with the use of hormonal contraceptives has now been shown in studies conducted in both Great Britain and the United States. Other risks, such as those of elevated blood pressure, liver disease, and reduced tolerance to carbohydrates, have not been quantitated with precision.

Long-term administration of both natural and synthetic estrogens in subprimate animal species in multiples of the human dose increases the frequency for some animal carcinomas. These data cannot be transposed directly to man. The possible carcinogenicity due to the estrogens can neither be affirmed nor refuted at this time. Close clinical surveillance of all women taking oral contraceptives must be continued.

INDICATION

Loestrin 1/20 is indicated for the control of conception.

CONTRAINDICATIONS

1. Thrombophlebitis, thromboembolic disorders, cerebral apoplexy, or a past history of these conditions.
2. Markedly impaired liver function.
3. Known or suspected carcinoma of the breast.
4. Known or suspected estrogen-dependent neoplasia.
5. Undiagnosed abnormal genital bleeding.
6. Pregnancy.

WARNINGS

1. The physician should be alert to the earliest manifestations of thrombotic disorders (thrombophlebitis, cerebrovascular disorders, pulmonary embolism, and retinal thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately.

Retrospective studies of morbidity and mortality in Great Britain and studies of morbidity in the United States have shown a statistically significant association between thrombophlebitis, pulmonary embolism, and cerebral thrombosis and embolism and the use of oral contraceptives. There have been three principal studies in Britain[1-3] leading to this conclusion, and one[4] in this country. The estimate of the relative risk of thromboembolism in the study by Vessey and Doll[3] was about sevenfold, while Sartwell and associates in the United States found a relative risk of 4.4, meaning that the users are several times as likely to undergo thromboembolic disease without evident cause as nonusers. The American study also indicated that the risk did not persist after discontinuation of administration, and that it was not enhanced by long continued administration. The American study was not designed to evaluate a difference between products. However, the study suggested that there might be an increased risk of thromboembolic disease in users of sequential products. This risk cannot be quantitated, and further studies to confirm this finding are desirable.

2. Discontinue medication pending examination if there is sudden partial or complete loss of vision, or if there is a sudden onset of proptosis, diplopia, or migraine. If examination reveals papilledema or retinal vascular lesions, medication should be withdrawn.

3. Since the safety of Loestrin 1/20 in pregnancy has not been demonstrated, it is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period.

4. A small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The long-range effect to the nursing infant cannot be determined at this time.

PRECAUTIONS

1. The pretreatment and periodic physical examinations should include special reference to breasts and pelvic organs, including Papanicolaou smear, since estrogens have been known to produce tumors, some of them malignant, in five species of subprimate animals.

2. Endocrine and possibly liver function tests may be affected by treatment with Loestrin 1/20. Therefore, if such tests are abnormal in a patient taking Loestrin 1/20, it is recommended that they be repeated after the drug has been withdrawn for two months.

3. Under the influence of estrogen-progestogen preparations, preexisting uterine fibromyomata may increase in size.

4. Because these agents may cause some degree of fluid retention, conditions which might be influenced by this factor, such as epilepsy, migraine, asthma, cardiac or renal dysfunction, require careful observation.

5. An alteration in menstrual patterns in many patients is likely to be induced by using Loestrin 1/20. The amount and duration of flow and cycle length may be variable. Because of these irregular bleeding patterns, it is important that the physician take this into consideration when attempting to determine if persistent bleeding is from organic causes.

6. Patients with a history of psychic depression should be carefully observed and the drug discontinued if the depression recurs to a serious degree.

7. Any possible influence of prolonged Loestrin 1/20 therapy on pituitary, ovarian, adrenal, hepatic, or uterine function awaits further study.

8. A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. The mechanism of this decrease is obscure. For this reason, diabetic patients should be carefully observed while receiving Loestrin 1/20 therapy.

9. The age of the patient constitutes no absolute limiting factor, although treatment with Loestrin 1/20 may mask the onset of the climacteric.

10. The pathologist should be advised of Loestrin 1/20 therapy when relevant specimens are submitted.

11. Susceptible women may experience an increase in blood pressure following administration of contraceptive steroids.

ADVERSE REACTIONS OBSERVED IN PATIENTS RECEIVING ORAL CONTRACEPTIVES

A statistically significant association has been demonstrated between use of oral contraceptives and the following serious adverse reactions:

Thrombophlebitis
Cerebral thrombosis
Pulmonary embolism

Although available evidence is suggestive of an association, such a relationship has been neither confirmed nor refuted for the following serious adverse reactions:

Neuro-ocular lesions, e.g. retinal thrombosis and optic neuritis

The following adverse reactions are known to occur in patients receiving oral contraceptives:

Nausea
Vomiting
Gastrointestinal symptoms (such as abdominal cramps and bloating)
Breakthrough bleeding
Spotting
Change in menstrual flow
Amenorrhea during and after treatment
Edema
Chloasma or melasma
Breast changes: tenderness, enlargement, and secretion
Change in weight (increase or decrease)
Changes in cervical erosion and cervical secretions
Suppression of lactation when given immediately postpartum
Cholestatic jaundice
Migraine
Rash (Allergic)
Rise in blood pressure in susceptible individuals
Mental depression

Although the following adverse reactions have been reported in users of oral contraceptives, an association has been neither confirmed nor refuted:

Anovulation post treatment
Premenstrual-like syndrome
Changes in libido
Changes in appetite
Cystitis-like syndrome
Headache
Nervousness
Dizziness
Fatigue
Backache
Hirsutism
Loss of scalp hair
Erythema multiforme
Erythema nodosum
Hemorrhagic eruption
Itching

The following laboratory results may be altered by the use of oral contraceptives:

Hepatic function: increased sulfobromophthalein retention and other tests

Coagulation tests: increase in prothrombin, factors VII, VIII, IX, and X.

Thyroid function: increase in PBI and butanol extractable protein-bound iodine, and decrease in T^3 uptake values

Metyrapone test

Pregnanediol determination

DOSAGE AND ADMINISTRATION

Special Notes on Administration

Menstruation usually begins two or three days, but may begin as late as the fourth or fifth day, after the brown tablets have been started. Because of the relatively low estrogenic content, Loestrin 1/20 is not a good cyclic regulator. There are patients whose inherent hormone balance will require larger amounts of estrogen to achieve cyclic regularity than that contained in Loestrin 1/20. These patients experience altered bleeding patterns, which do not conform to treatment schedules, while taking the white Loestrin 1/20 tablets. However, it is important that patients adhere to the dosage schedule regardless of when bleeding occurs.

The occurrence of altered bleeding is highest in Cycle 1. The majority of patients will have seven days or less of total bleeding during the 28-day cycle (including both withdrawal bleeding and breakthrough bleeding and spotting). Should irregular bleeding occur, patients should be reassured and instructed to continue taking the tablets as directed. If by the third cycle the irregular patterns are unacceptable, consideration should be given to changing the medication to a product with higher estrogen content (Norlestrin®-1 mg or Norlestrin-2.5 mg). The physician should be alert to the fact that the irregular bleeding patterns could mask bleeding from organic causes, and appropriate diagnostic measures should be taken if the bleeding persists or continues after changing to a higher estrogen-content product.

SE

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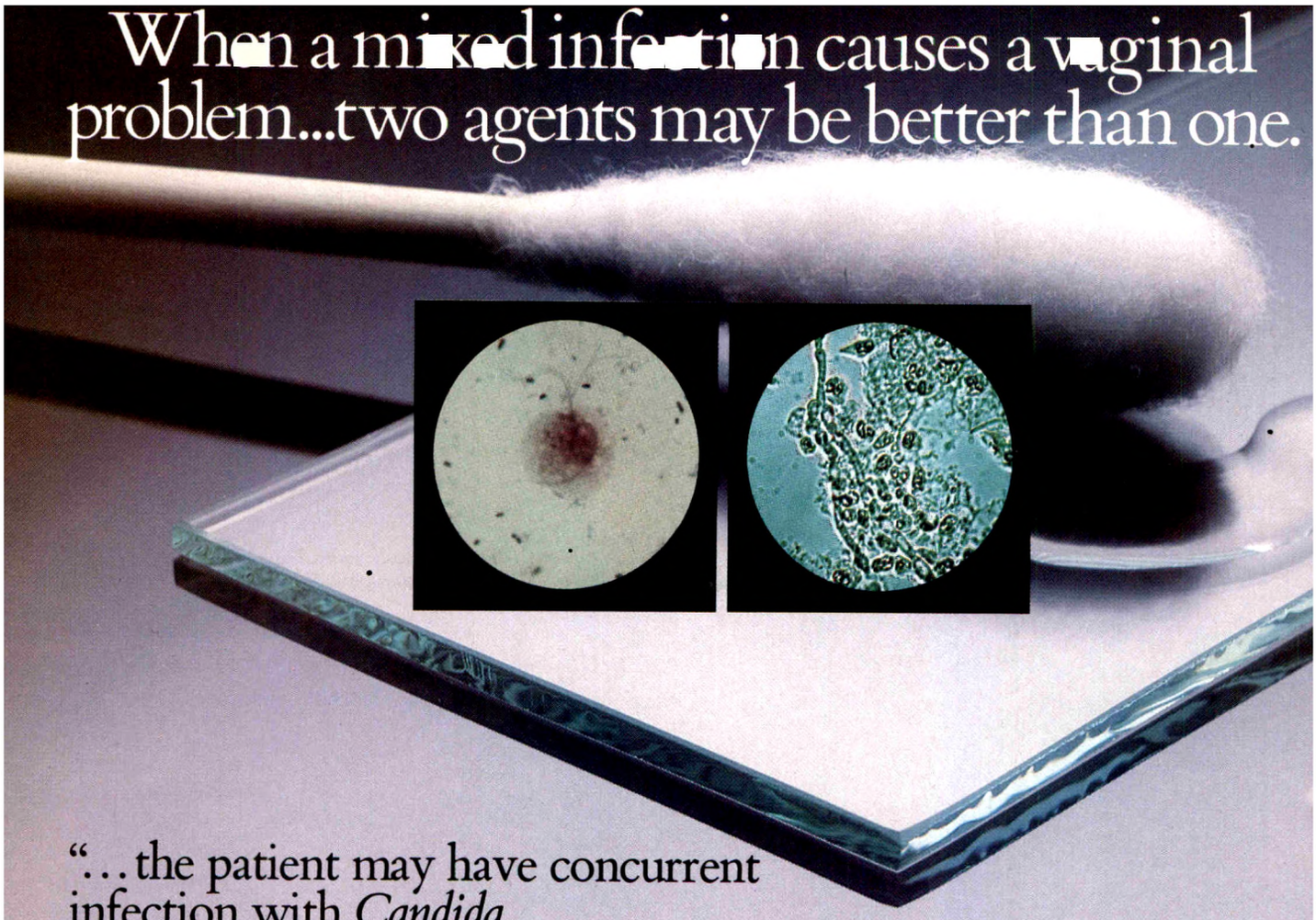
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Flagyl® (metronidazole) is the recognized treatment of choice for trichomonal vaginitis, which is often characterized by a bubbly, thin, yellow to light green or grey discharge. In addition, secondary symptoms of pruritus vulvae, soreness, irritation, burning, dyspareunia, offensive odor and, occasionally, urinary frequency may occur. The systemic action of Flagyl may usually be relied upon to eliminate trichomonads from the vagina, the paraurethral and Bartholin's glands, and the bladder as well.

Therapy of choice for women: One 250-mg. Flagyl tablet orally three times daily for ten days in conjunction with treatment of her male partner, when he has been shown to harbor the organism.

The only choice for infected men: One 250-mg. Flagyl tablet orally two times daily for ten days concurrent with treatment of his infected female partner. Topical medications are useless in men. Only the systemic action of Flagyl can eliminate trichomonads entrenched in the male genitourinary tract.

1. Cibley, L. J. and Kasdon, S. C.: A New Look at Vulvovaginitis, ed. 2, Chicago, Searle & Co., 1972, p. 11.

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Indications: For the treatment of trichomoniasis in both male and female patients and in the sexual partners of patients with a recurrence of the infection provided trichomonads have been confirmed by appropriate laboratory procedures (wet smear or culture). The oral tablets are indicated also for acute intestinal amebiasis (amebic dysentery) and amebic liver abscess.

Contraindications: Evidence or history of blood dyscrasia, active organic disease of the CNS, the first trimester of pregnancy and a history of hypersensitivity to metronidazole.

Warnings: Flagyl should not be used in the first trimester of pregnancy (see *Contraindications).* Use with discretion during the second and third trimesters of pregnancy and restrict to those pregnant patients not cured by topical measures. Flagyl (metronidazole) is secreted in the breast milk of nursing mothers. It is not known whether this can be injurious to the newborn.

Note: Tumorigenicity Studies in Rodents. Metronidazole has shown evidence of tumorigenic activity in a number of studies involving chronic, oral administration in mice and rats.

Most prominent among the effects in the mouse was the promotion of pulmonary tumorigenesis. This has been observed in all five reported studies in that species, including one study in which the animals were dosed on an intermittent schedule (administration during every fourth week only). The published results of one of the mouse studies indicate increases in the incidence of malignant lymphomas as well as pulmonary neoplasms associated with lifetime feeding of the drug. All these effects are statistically significant.

Two long-term toxicity studies in the rat have been completed. There was a statistically significant increase in the incidence of various neoplasms, particularly mammary tumors, among female rats administered metronidazole over that noted in the concurrent female control groups.

Unpublished results of a lifetime tumorigenicity study in hamsters have been reported to be negative.

Precautions: Mild leukopenia has been reported during Flagyl use; total and differential leukocyte counts are recommended before and after treatment with the drug, especially if a second course is necessary. Avoid alcoholic beverages during Flagyl therapy because abdominal cramps, vomiting and flushing may occur. Discontinue Flagyl promptly if abnormal neurologic signs occur. Exacerbation of moniliasis may occur. In amebic liver abscess, aspirate pus during metronidazole therapy.

Adverse Reactions: Nausea, headache, anorexia, vomiting, diarrhea, epigastric distress, abdominal cramping, constipation, a metallic, sharp and unpleasant taste, furry tongue, glossitis and stomatitis possibly associated with a sudden overgrowth of *Monilia,* exacerbation of vaginal moniliasis, an occasional reversible moderate leukopenia, dizziness, vertigo, incoordination and ataxia, numbness or paresthesia of an extremity, fleeting joint pains, confusion, irritability, depression, insomnia, mild erythematous eruptions, "weakness," urticaria, flushing, dryness of the mouth, vagina or vulva, pruritus, dysuria, cystitis, a sense of pelvic pressure, dyspareunia, fever, polyuria, incontinence, decrease of libido, nasal congestion, proctitis, pyuria and darkened urine have occurred in patients receiving the drug. Patients receiving Flagyl may experience abdominal distress, nausea, vomiting or headache if alcoholic beverages are consumed. The taste of alcoholic beverages may also be modified. Flattening of the T wave may be seen in ECG tracings.

Dosage and Administration: For Trichomoniasis. *In the female:* One 250-mg. tablet orally three times daily for ten days. Courses may be repeated if required in especially stubborn cases; in such patients an interval of four to six weeks between courses and total and differential leukocyte counts before, during, and after treatment are recommended. Vaginal inserts of 500 mg. are available for use, particularly in stubborn cases. *When the vaginal inserts are used,* one 500-mg. insert is placed high in the vaginal vault each day for ten days and the oral dosage is reduced to two 250-mg. tablets daily during the ten-day course of treatment. Do not use the vaginal inserts as the sole form of therapy. *In the male:* Prescribe Flagyl only when trichomonads are demonstrated in the urogenital tract, one 250-mg. tablet two times daily for ten days. Flagyl should be taken by both partners over the same ten-day period when it is prescribed for the male in conjunction with the treatment of his female partner.

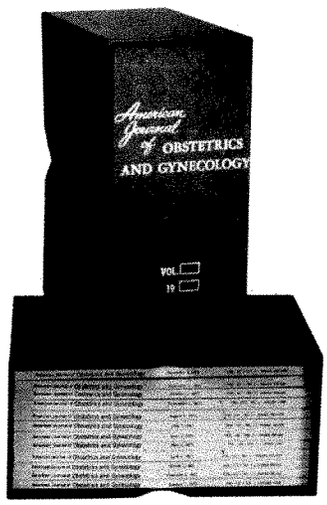
For Amebiasis. *Adults:* For acute intestinal amebiasis, 750 mg. orally three times daily for 5 to 10 days. For amebic liver abscess, 500 to 750 mg. orally three times daily for 5 to 10 days. *Children:* 35 to 50 mg./kg. of body weight/24 hours, divided into three doses, orally for ten days.

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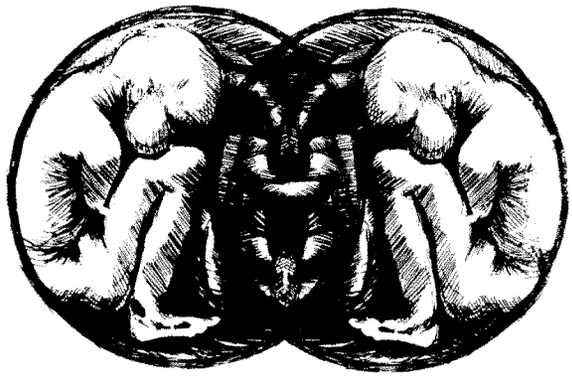
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THE DEPARTMENTS OF OBSTETRICS AND GYNECOLOGY OF THE FACULTIES OF MEDICINE OF THE UNIVERSITIES OF THE PROVINCE OF QUEBEC

ANNOUNCE:

A SYMPOSIUM ON FETAL MONITORING

The Symposium will be held at the Château Frontenac in Quebec City, Canada, from Wednesday, April 21st to Saturday, April 24th 1976. The three days will be devoted to the study of recent advances in monitoring the fetal heart and uterine contraction. Internationally known experts in these fields will be giving presentations. Lectures and workshops have been organised for physicians and nurses interested in the subjects.

Official languages will be English and French with simultaneous translation available.

Registration will be closed April 1st 1976. The regular fee will be: physicians, one hundred and fifty canadian dollars ($150.00); nurses, fifty dollars ($50.00), including three breakfasts and two lunches at the Château Frontenac. For registration after April 1st, the fee will be: physicians, two hundred dollars ($200.00); nurses, seventy-five dollars ($75.00). Cheques should be made payable to: Symposium de monitorage foetal, Département d'Obstétrique-Gynécologie, Faculté de Médecine, Cité Universitaire, Québec, Canada, G1K 7P4.

For further information write to: Adrien Bastide M.D., Hôpital Saint-Francois d'Assise, 10 de l'Espinay, Québec, Canada, G1L 2H1 or call (418) 688-8710.

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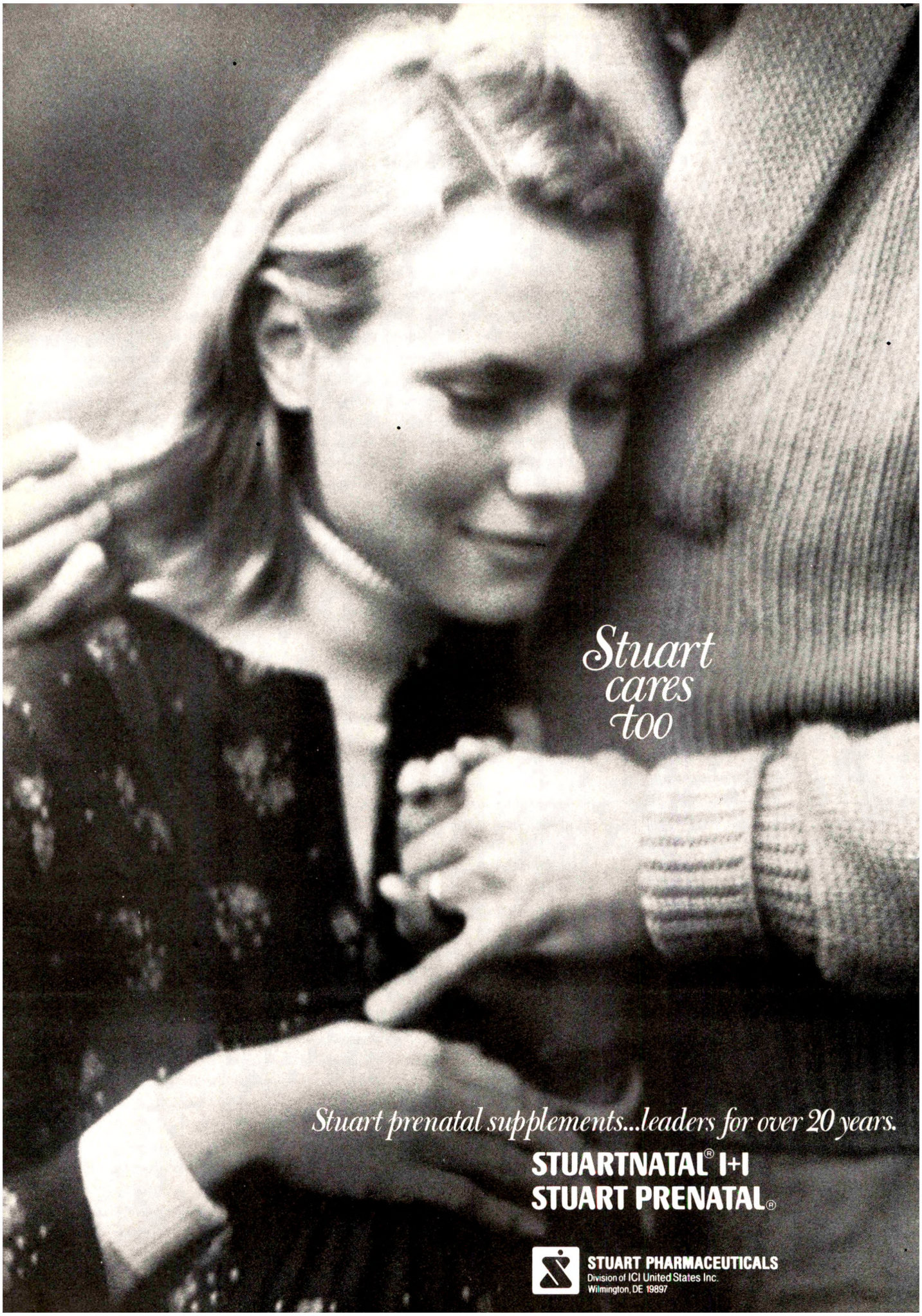
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CURRENT INVESTIGATION

This section offers prompt first announcement of new observations or discoveries. Articles should be limited to 1,500 words and 6 references. Illustrations or additional references require a proportionate reduction in total words.

The inhibition of pulmonary maturation in the fetal rabbit by maternal treatment with phenobarbital

EDWARD H. KAROTKIN, M.D.
MASUMITSU KIDO, M.D.
RALPH REDDING, M.D.
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Providence and Pawtucket, Rhode Island, and Lake Placid, New York

Phenobarbital was administered to pregnant rabbits, 7 to 10 days before the delivery of their fetuses at 27 to 30 days of gestation. There were no differences in body weight or wet or dry lung weights between control animals and phenobarbital-treated pups at similar gestational ages. The phospholipid content (PLC) of the alveolar wash was lower in the phenobarbital-treated group, but there was no difference in the lung tissue PLC between the treated and control groups. Phenobarbital-treated pups had higher opening pressures and fewer lamellar bodies than the control animals. These data suggest that phenobarbital may have an inhibitory effect on surfactant production and/or release.

From the Department of Pediatrics, Women and Infants Hospital of Rhode Island, and the Department of Medicine, Memorial Hospital, Pawtucket, Brown University Program in Medicine, W. Alton Jones Cell Science Center, Lake Placid

Supported in part by Grants H.L. 17110-01 and H.L. 15266 from the American Lung Association, National Heart and Lung Institute.

Reprint requests: Dr. William Oh, Women and Infants Hospital of Rhode Island, 50 Maude St., Providence, Rhode Island 02908.

PREVIOUS STUDIES have shown that maternal administration of corticosteroids can induce fetal lung maturation in animal species.[1, 2] Subsequent studies by Liggins and Howie[3] also demonstrated an acceleration of fetal lung maturation in human infants indicated by a reduction in the incidence of hyaline membrane disease in those babies whose mothers were treated with betamethasone, 48 to 72 hours prior to delivery.

Since phenobarbital is known to be a potent inducer

Table I. Alveolar wash and lung tissue phospholipid contents in phenobarbital-treated rabbit pups

Gestational age (days)	*Alveolar wash phospholipid content (μg/mg. dry weight)*		*Lung tissue phospholipid content (μg/mg. wet weight)*	
	Control	*Phenobarbital-treated*	*Control*	*Phenobarbital-treated*
27	7.0 ± 1.6 (10/4)	11.1 ± 3.1 (8/3)	357.8 ± 8.8 (13/4)	341.1 ± 8.3 (9/3)
28	54.7 ± 8.7 (19/6)	16.9 ± 2.2* (11/3)	367.5 ± 12.1 (10/6)	370.5 ± 10.2 (11/3)
29	75.5 ± 7.7 (13/4)	49.0 ± 4.8* (11/6)	475.3 ± 24.0 (16/4)	433.3 ± 14.6 (12/6)
30	116.8 ± 16.8 (16/6)	83.4 ± 7.1 (7/3)	593.1 ± 18.6 (14/6)	546.1 ± 19.9 (9/3)

Values given are means ± standard errors. Numbers in parentheses are the numbers of fetuses/numbers of litters.
*$p < 0.01$ when compared to control animals of similar gestational ages.

Table II. Opening pressure (cmH_2O) of fetal lung tissues in phenobarbital treated rabbit pups

Gestational age (days)	*Control*	*Phenobarbital-treated*
27	30 ± 2 (12)	26 ± 2 (12)
28	20 ± 1 (28)	26 ± 1* (15)
29	19 ± 2 (27)	24 ± 2* (17)
30	14 ± 1 (25)	17 ± 2 (15)

Values given are means ± standard errors. Numbers in parentheses are the numbers of fetuses. The numbers of litters are shown in Table I.
*$p < 0.01$ when compared to control animals of similar gestational ages.

of enzyme activity,[4] we had initially studied this pharmacologic agent for its possible accelerating effect on fetal lung maturation. However, the results we obtained and reported in this communication were contrary to our expectations—i.e., we observed an inhibitory rather than a stimulatory effect of phenobarbital on fetal lung maturation.

Material and methods

Thirty-five time-dated pregnant New Zealand rabbit does (20 control and 15 phenobarbital-treated animals) were the subjects of this study. The does were treated at 20 days' gestation with intramuscular administration of normal saline (0.5 ml. per dose) or phenobarbital (12.5 ml. per kilogram per dose) twice daily until the time of delivery (e.g., those born at 27 days of gestation received 7 days of treatment). The does were fed ad libitum with water and Purina chow, and daily observations were made to assure that the phenobarbital-treated does were not sedated. The maternal weight gain during the study periods was comparable between saline- and phenobarbital-treated groups.

The pups were delivered by hysterotomy at 27, 28, 29, and 30 days of gestation with the use of intravenous pentobarbital anesthesia. Each pup was weighed, and a tracheostomy was performed on approximately one half of the litter. After placement of a tracheal cannula, the head and trunk below the diaphragm were removed, with the lungs left intact within the thoracic cavity. An anterior section of diaphragm was removed to allow direct pressure access to the lungs. The lung preparation was suspended in a sealed glass chamber, and 9 to 19 successive pressure-volume curves were obtained until there were several stable and reproducible loops.[5] The opening pressure was calculated from one of the most reproducible pressure-volume curves.

The alveolar wash materials were obtained by washing the tracheobronchial tree with 1 to 2 ml. of normal saline 10 times. The wash was centrifuged at 300 × g for 10 minutes to remove cellular debris, and the supernate was centrifuged at 16,000 × g for one hour to obtain the surfactant pellet. The pellet was dissolved in a chloroform:methanol (2:1) mixture and subsequently analyzed for phospholipid content.[6] The lungs from the remaining 3 or 4 pups were removed en bloc and homogenized in a Teflon grinder. Similar extraction and phospholipid analysis procedures were performed on the lung tissue homogenate. Dry and wet lung weights were obtained for the purpose of unit expression of alveolar wash and tissue homogenate phospholipids, respectively. The lungs from 11 fetuses of 29 days' gestation, (six control fetuses from three litters and five phenobarbital-treated fetuses from four litters) were prepared for electron microscopy with conventional techniques.[6] Type II cells were examined morphologically, and the mean lamellar body count from 25 type II cells in each fetus was determined by one of us (W. D.) who was unaware of the group source of the lung tissue.

Results

The body weights and fresh and dry lung weights were similar in the control and phenobarbital-treated groups. As shown in Table I, the alveolar wash phospholipid content in the phenobarbital-treated litters was significantly lower at 28 and 29 days of gestation. There was no significant difference in the

phospholipid content in the lung tissue homogenate between the control and phenobarbital-treated groups at all gestational ages. Table II shows the significant difference in opening pressure between phenobarbital-treated and control litters at 28 and 29 days of gestation. The higher value for opening pressure in the phenobarbital-treated group is statistically significant ($p < 0.01$). There were significantly fewer lamellar bodies per type II cell (mean ± standard error) at 29 days' gestation in the phenobarbital-treated group when compared to the saline-treated control group, (5.2 ± 0.4 versus 7.2 ± 0.5, respectively; $p < 0.01$).

Comment

The process of lung maturation involves the anatomic development of the alveoli, ingrowth and proliferation of blood vessels into the alveolar areas, the development of cuboidal epithelium into flattened cells lining the alveoli, and the specialization of type II alveolar cells for the production of surfactant. It is known that the phospholipid content of the alveolar fluid correlates directly with the functional maturation of the lung. The reduction in the number of lamellar bodies in the type II cells, together with the reduced alveolar wash phospholipid content and the higher opening pressure in the phenobarbital-treated pups, would suggest that phenobarbital may have an inhibitory effect on the synthesis and release of surfactant by the fetal lung. However, our data do not distinguish whether the effect is primarily on synthesis or on release of the surfactant or both.

It has been shown that phenobarbital stimulates hepatic microsomal enzyme systems, resulting in an increase in oxidation and conjugation of endogenous corticosteroids.[7] It seems probable that acceleration of steroid metabolism and degradation might be the mechanism which accounts for the inhibitory effect of phenobarbital on lung maturation. On the basis of this study, it may be appropriate to re-examine the role of phenobarbital administration to pregnant women with specific reference to the possibility of inhibitory effects on fetal lung maturation.

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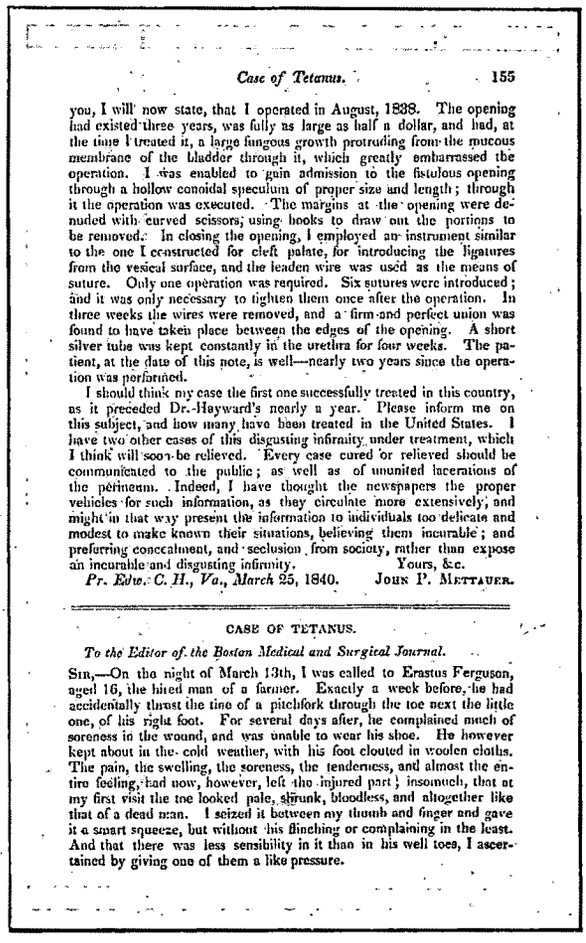
OBSTETRICS AND GYNECOLOGY

Lawrence D. Longo, M.D.

Vesico-vaginal fistula

JOHN PETER METTAUER

The Boston Medical and Surgical Journal, vol. 22, pp. 154-155, 1840



Case of Tetanus. 155

you, I will now state, that I operated in August, 1838. The opening had existed three years, was fully as large as half a dollar, and had, at the time I treated it, a large fungous growth protruding from the mucous membrane of the bladder through it, which greatly embarrassed the operation. I was enabled to gain admission to the fistulous opening through a hollow conoidal speculum of proper size and length; through it the operation was executed. The margins at the opening were denuded with curved scissors; using hooks to draw out the portions to be removed. In closing the opening, I employed an instrument similar to the one I constructed for cleft palate, for introducing the ligatures from the vesical surface, and the leaden wire was used as the means of suture. Only one operation was required. Six sutures were introduced; and it was only necessary to tighten them once after the operation. In three weeks the wires were removed, and a firm and perfect union was found to have taken place between the edges of the opening. A short silver tube was kept constantly in the urethra for four weeks. The patient, at the date of this note, is well—nearly two years since the operation was performed.

I should think my case the first one successfully treated in this country, as it preceded Dr. Hayward's nearly a year. Please inform me on this subject, and how many have been treated in the United States. I have two other cases of this disgusting infirmity under treatment, which I think will soon be relieved. Every case cured or relieved should be communicated to the public; as well as of ununited lacerations of the perineum. Indeed, I have thought the newspapers the proper vehicles for such information, as they circulate more extensively, and might in that way present the information to individuals too delicate and modest to make known their situations, believing them incurable; and preferring concealment, and seclusion from society, rather than expose an incurable and disgusting infirmity. Yours, &c.

Pr. Edw. C. H., Va., March 25, 1840. JOHN P. METTAUER.

CASE OF TETANUS.

To the Editor of the Boston Medical and Surgical Journal.

SIR,—On the night of March 13th, I was called to Erastus Ferguson, aged 16, the hired man of a farmer. Exactly a week before, he had accidentally thrust the tine of a pitchfork through the toe next the little one, of his right foot. For several days after, he complained much of soreness in the wound, and was unable to wear his shoe. He however kept about in the cold weather, with his foot clouted in woolen cloths. The pain, the swelling, the soreness, the tenderness, and almost the entire feeling, had now, however, left the injured part; insomuch, that at my first visit the toe looked pale, shrunk, bloodless, and altogether like that of a dead man. I seized it between my thumb and finger and gave it a smart squeeze, but without his flinching or complaining in the least. And that there was less sensibility in it than in his well toes, I ascertained by giving one of them a like pressure.

Prior to the 1850's, the relief of vesico-vaginal fistula was one of the greatest stumbling blocks in gynecology. Mettauer, a rural Virginian physician, was apparently the first to successfully close a vesico-vaginal fistula in America (August, 1838), although George Hayward first reported his case which occurred the following year. Key elements of his success were: denuding the margins of the "half-a-dollar"–sized opening, closing it with six lead wire sutures, and leaving a silver retention catheter in place for four weeks. In all, he successfully repaired six such cases (Am. J. Med. Sci. 14: 117, 1847). He was also the first in this country to successfully repair a lacerated perineum with a rectovaginal fistula. An indefatigable surgeon, he pioneered operations for urethral stricture, hypospadias, vesicle calculi, cleft palate, and cataracts. (Photograph courtesy of the National Library of Medicine.)

CURRENT DEVELOPMENTS

Alpha-fetoprotein

H. LORRIN LAU, M.D., M.P.H.
SUSAN E. LINKINS, B.A.
Baltimore, Maryland

Despite all the information about AFP presented, a great deal still needs to be discovered, especially in pregnancy. Geographical and racial differences remain to be elucidated. Similarly, differences in obstetric population in Helsinki and Baltimore, for example, are only now being studied. Sex differences may exist; Lardinois and associates[195] cited a higher level of AFP in male than female fetuses. The important questions that need to be answered are whether AFP assays can help improve the other half of prenatal care—that directed to the fetus—and whether the AFP model can help enhance our understanding of the similarities and differences of fetal and cancer cells.

A FASCINATING substance, alpha-fetoprotein (AFP), which is useful in pregnancy as a means of monitoring fetal development and in cancer as a model for chemotherapy, is the subject of this article. The growing interest in the biology and uses of AFP in pregnancy and cancer is shown by the increase in published articles on AFP—from 160 before 1970 to 337 after 1970.

In 1944, Pedersen[1] described fetuin, a glycoprotein in calf serum, and gave substantive birth to the new concept that there are unique substances in the fetus which are absent in the normal adult. These substances reappear in adults only under unusual circumstances, such as malignancy. Fetuin, however, is different from AFP.[2] In 1956, Bergstrand and Czar[3] reported a new fraction in the electrophoresis of plasma proteins migrating between the albumin and alpha-$_1$ globulin fraction in a fetus a few weeks old which was not present in maternal serum. They called it a "new protein fraction" or "x component." Its proximity to the band of albumin (see Fig. 1) is probably the reason why the existence of this substance was hidden for so long. Others who saw this unique fraction include Halbrecht and Klibanski,[4] Galdo and associates,[5] Bodman,[6] de Muralt and Roulet,[7] Andreoli and Robbins,[8] and Tatarinov.[9] In 1965, Masopust and Kotal[10] called it "fetoprotein." In 1964, Tatarinov and Abelev wrote about alpha and beta embryo-specific antigen components and, to distinguish Bergstrand and Czar's protein, David Gitlin arbitrarily labeled it alpha-fetoprotein.[11, 12] Gitlin's label was adopted as the official name of this substance by the International Agency for Research on Cancer in 1959.[13] Synonyms which have been applied at one time or another include alpha$_1$-embryo-specific protein, protein F, alpha-$_1$-fetoprotein, and ESA globulin. Since 1969, it has been abbreviated as "AFP."

The clinical importance of AFP has stimulated interest in three main areas. In biology, it has provided insight into a relatively new concept of fetospecific proteins, transient proteins, transitory cell antigens,[14] embryo-specific proteins,[15] or what in the future may

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Reprint requests: Dr. H. Lorrin Lau, The Johns Hopkins University, School of Medicine, 725 North Wolfe St., Baltimore, Maryland 21205

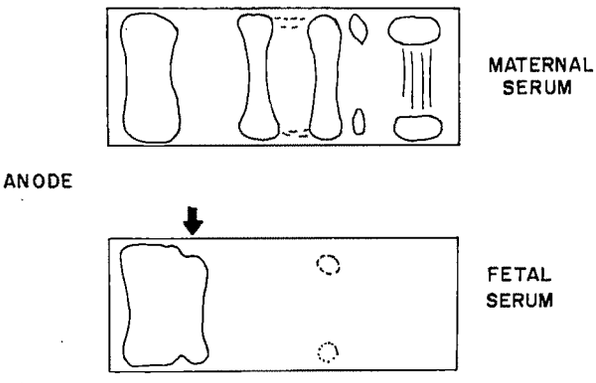


Fig. 1. Diagrammatic sketch of separation of AFP from albumin with cellulose acetate electrophoresis in human maternal serum and fetal serum. Arrow indicates AFP next to albumin. (Adapted from Gitlin and Boesman.[21])

be called "developmental proteins" because they may be specific to the placenta rather than to the fetus or to both. In oncology, AFP and its antibody are currently providing one of the best models for the study of cancer therapy directed specifically against AFP-producing cells. This is not limited to hepatocytes, as AFP-producing cells have now been described in a variety of tumors. One of the most fascinating areas, however, is the use of AFP assays in antenatal diagnosis, not only of neural tube defects but of a wide variety of prenatal conditions. Unlike most fetal monitors, which are restricted to a small portion of gestation, measurement of AFP is clinically useful in all three trimesters for a variety of indications.

Biology, chemistry, physiology, and immunology

Biology.

Embryo-specific or developmental substances. Fetospecific proteins, transient proteins, transitory cell antigens, and embryo-specific proteins all refer to proteins or glycoproteins which are synthesized only during a particular phase of growth. These disappear or persist only in trace amounts during adult life, unless their synthesis is restimulated by some unusual condition such as injury, infection, or a malignancy. The term "developmental substances" or "embryonic substances" is preferable because this now appears to be a general biologic concept, including pure proteins along with glycoproteins and carbohydrates and substances produced by the placenta or fetus or both.

Fetuin[2, 16, 17] differs from AFP in physicochemical properties, molecular weight, carbohydrate content, and amino acid composition. Moreover, AFP exists with fetuin in calf serum but shows no immunologic cross-reaction.[2, 18] Fetuin may not be embryo-specific, as originally thought, as it has now been found in the adult cow, which is not true for calf AFP.[19]

Human AFP migrating in the alpha$_2$ globulin region has antigenic determinants in common with homologous AFP as described in at least 18 species[11, 18, 20] including the squirrel, monkey, cat, dog, seal, sheep, and armadillo.[21]

A convenient classification of developmental substances or transitory cell antigens[14, 22] used by Masseyeff[19] has three major groups: circulating proteins, enzymes, and intracellular substances. This classification easily shows the difference between a cellular constituent like CEA (carcinoembryonic antigen) and a secreted protein like AFP.[23, 24] Circulating proteins include fetal hemoglobin,[25] beta fetoprotein,[26] HCG, and alpha$_2$-H globulin.[27, 28] Alpha-$_2$H globulin is of particular interest because it has properties similar to those of AFP. Buffe and Rimbaut[27] have shown that it is a glycoferroprotein which is immunologically identical with ferritin, synthesized by the liver, and present in larger amounts in the fetus than in the adult. Like AFP, it has immunosuppressive activity and inhibits mitogen and antigenic lymphoblastic transformation. Enzymes or isoenzymes include: aldolase,[29] alkaline phosphatase,[22, 30] desoxycytitidylate diaminase,[14] glucosamine-6-phosphate synthetase,[31] glycogen phosphorylase isoenzymes,[32] glutaminase, hexosaminidase,[29] LDH, pyruvate kinase,[29, 33] thymidine kinase,[34] and thymidylate synthetase.[35] This is at best a conservative list, which merely serves to illustrate the rapidly increasing list of developmental substances. A new branched-chain amino transferase isoenzyme, for example, has been recently reported by Ichihara.[36] Intracellular substances which circulate in the serum (sometimes only in low concentrations) include CEA,[23] FSG (feto-sulfo glyco-protein),[37] FGA (fetal gut antigen),[38] and the isoferritins recently discussed by Drysdale and associates.[39]

In obstetrics, it is clinically useful to differentiate between assays which measure fetal substances like AFP and those which measure placental substances like HPL (human placental lactogen). Frequently, measurement of HPL is regarded as a reflection of fetal status when in fact it is a reflection of placental status. The point is that terminology applied in the future should have clinical connotations in addition to biochemical denotations to be clinically useful.

Evolutionary trend. In the evolutionary scheme, Gitlin[40] calculated that AFP may have been present in primitive forms like the shark over 400 million years ago. Synthesized from the entoderm of the embryonic foregut, AFP "early in evolution" arose mainly from the gastrointestinal tract. With time, synthesis shifted

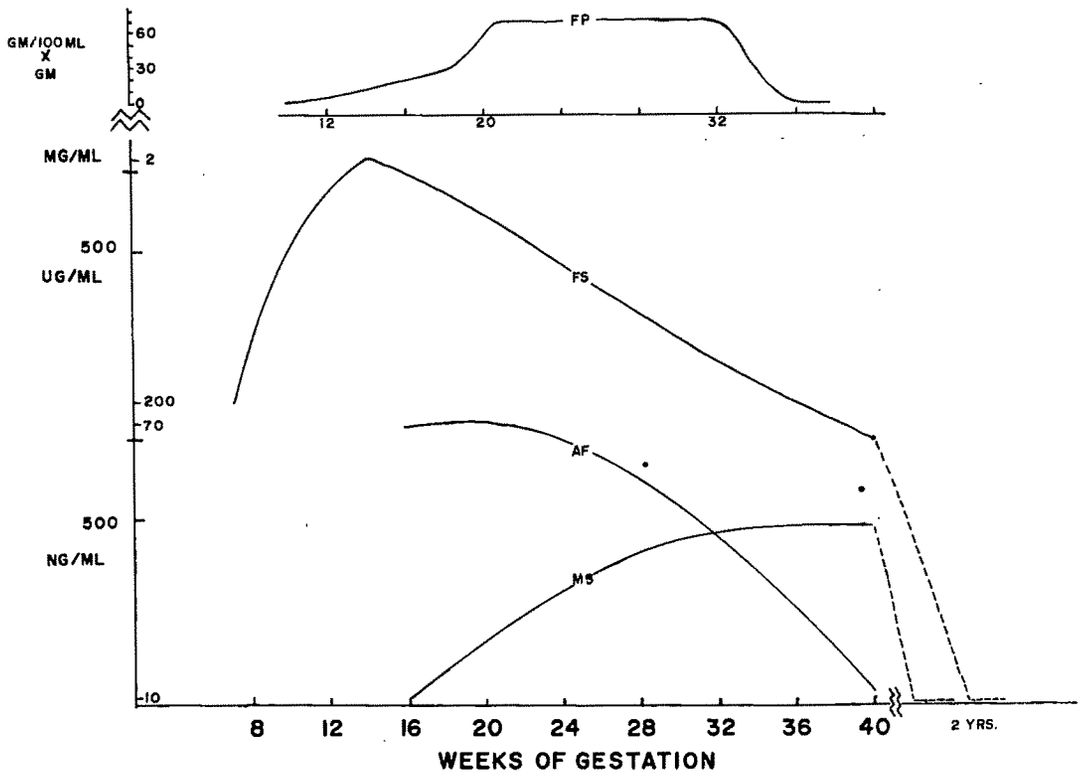


Fig. 2. Concentrations of AFP in fetal serum (FS), material serum (MS), and amniotic fluid (AF) and fetal production (FP) during pregnancy. (Modified from Gitlin[42]).

away from the stomach to outgrowths of the entoderm, liver, and yolk sac.

In the shark, AFP is produced mainly in the stomach, with lesser amounts in the intestinal mucosa and liver and with little production in the yolk sac. In birds, most AFP synthesis occurs in the yolk sac, with no synthesis in the liver. In mammals, AFP synthesis is greatest in the liver, followed by the yolk sac, with the least amount produced in the gastrointestinal tract.

Ontogeny in human beings. In human beings, at 4 to 8 weeks of gestation, the yolk sac rivals the fetal liver in AFP production. As the yolk sac degenerates at 11.5 weeks, the liver overtakes the yolk sac in the production of AFP. The gastrointestinal tract also produces AFP, but in small amounts; the kidney or placenta may possibly produce trace amounts.[43]

Synthesis by the liver is responsible for the rise in the fetal serum concentration from 70 μg per milliliter at 6.5 weeks to 2,000 μg per milliliter at 9.5 weeks. The fetal serum concentration reaches a peak at 14 weeks with 2,000 to 3,000 μg per milliliter of AFP. Perfusion studies by Kekomaki and associates[41] have shown that human fetal livers release 19 to 26 μg per minute of AFP.

After the fourteenth week of gestation, the fetal serum concentration falls to levels of 13 to 86 μg per milliliter at birth (Fig. 2). While the fetal serum concentration declines, the hepatic production of AFP increases to a peak at 20 weeks and remains fairly constant until 32 weeks. This decrease in fetal concentration is due to a disproportionately large increase in fetal growth and volume of body fluid rather than to a decrease in AFP hepatic synthesis. After the thirty-second week, the liver production drops sharply and fetal serum concentrations also fall. This is shown in Fig. 2.

IN THE MOTHER. At the peak of fetal serum concentrations of AFP, maternal serum levels are slightly raised above normal adult nonpregnant levels. Maternal serum peaks at 450 ng. per milliliter at 32 weeks, at which time the fetal concentration is 200,000 ng. per milliliter, a 40-fold difference. A concentration gradient from fetus to mother obviously exists, but the actual amount transferred is small—probably less than 450 μg per day. This transfer is due mainly to increased placental permeability, not increased placental area or increased AFP concentration in the conceptus. AFP can also be transferred from the fetus to the mother via the amniotic fluid. The total amount transferred is only 6 per cent of the

amount needed to maintain the maternal serum level at 450 ng. per milliliter. This is based on an amniotic fluid volume of 800 ml. at 32 weeks, with AFP concentration of 15 μg per milliliter. It is known that 0.23 per cent of proteins present in amniotic fluid pass into the maternal pool each day.[42].

IN AMNIOTIC FLUID. AFP accounts for less than 0.5 per cent of total proteins present in the amniotic fluid, yet nearly all AFP is of fetal origin. Thus, in a neural tube defect with a ten-fold increase in fetal serum proteins, AFP concentrations in amniotic fluid will increase 10 times while the total protein hardly changes. If total protein in amniotic fluid does rise, then serious fetal compromise must be considered, as 60 per cent of all protein is cleared daily by fetal swallowing. For this reason, total protein in amniotic fluid should be measured along with AFP in abnormal situations to separate the activities of the two types of proteins. Alpert of Harvard proposed this practice after analysis of over 500 samples of amniotic fluid.[42]

In the newborn infant, serum concentrations range from 13 to 86 μg per milliliter at birth and decline rapidly with a half-life of 3½ days to negligible amounts of 1 to 2 ng. per milliliter by 2 years of life.

In a study of postnatal levels of AFP in 200 children from 2 weeks to 15 years of life, Masseyeff and associates[43] found a high scatter of levels ranging from 10 to 150 μg per milliliter in newborn infants with less scatter of levels by 2 or 3 years of age. This suggested that the mechanism regulating the level of blood AFP becomes more stable with increasing age: Gitlin attributed the high scatter of values at birth to the difference that 2 weeks of gestation can make on AFP levels.

In the normal adult, serum AFP levels are 1/20,000th of fetal serum levels at birth, or less than 10 to 23 ng. per milliliter.[44]

Half-life. The half-life of AFP seems to vary with the clinical condition. Alpert[45] reported a half-life of 2.0 days or less following hepatectomy; Mukojima and associates[46] reported a half-life of 3.7 days following surgical treatment for hepatoma and hepatoblastoma; Gitlin[42] reported a half-life of 3.5 days in newborn plasma; Seppälä[47] reported a half-life of 4 to 5 days following delivery or abortion. Of clinical importance is the fact that the half-life of AFP is measured in days, in contrast to the half-life of other biochemical fetal measures, such as HPL, whose half-life is measured in minutes. This is important when considering the combined use of AFP and HPL measurements for the prenatal assessment of multiple gestation and prediction of pregnancy outcome in a threatened abortion.[48, 49]

Chemistry.

Physicochemical properties. The physicochemical properties of AFP have been investigated by Gitlin and Boesman,[11] Masopust and associates,[50] Nishi,[51] Nishi and Hirai,[52], Ruoslahti and associates,[53, 54] and Alpert and associates.[55] AFP has a sedimentation constant of 4.5 to 5, a molecular weight of approximately 70,000, an isoelectric pH of 4.75 to 5.08, an electrophoretic mobility of 6.08×10^{-5} $cm.^2\text{-}V.^{-1}sec.^{-1}$, a carbohydrate content of 4.3 per cent consisting of hexose, hexosamine, and sialic acid, a 3 per cent nonnitrogenous sugar content, a nitrogen content of 14.7, and a sulfur content of 1.7. It was believed to be a single polypeptide chain by Ruoslahti and associates.[54] Thus, AFP is a distinct chemical entity.

Similarity between fetal liver and hepatoma. The AFP's from hepatoma and normal fetal liver appear to have identical physicochemical properties according to Nishi[51] and Ruoslahti and associates.[54].

Microheterogeneity. Different forms of AFP have been described by Alpert and associates[55] and Watanabe and associates.[56] Watanabe described the microheterogeneity of rat AFP on polyacrylamide disc electrophoresis following treatment with neuraminidase. He found that AFP_a has 2.5 molecules of sialic acid per molecule and AFP_b has 4.5 molecules of sialic acid per molecule of AFP. These sialic acid residues do not contribute to the immunogenicity of AFP. Alpert[57] reported at least three different AFP isoproteins. Two forms are immunologically identical, whereas the third does not precipitate a monospecific antibody to AFP. This third form may be responsible in part for differing RIA values. Variance of AFP has also been reported by Purves and associates.[58, 59]

Distribution. Abelev[15] pointed out that AFP is the predominant protein in the sera of mice and rats in the earliest stages of development. As in the human embryo, AFP prevails over albumin until 8 weeks of gestation, when both substances are present at comparable concentrations.[60] The decrease in AFP level, according to Abelev,[15] is due to a smaller number of cells synthesizing AFP rather than a diminution of synthesis in all cells. On immunofluorescence, brightly luminescent cells distributed around central veins of the liver appeared different from the majority of liver cells. These diminished in number coincident with a decline in AFP production. Thus, in the fetal liver, hepatomas, and rapidly regenerating livers following CCl_4 treatment, only certain cells produce AFP.

Immunofluorescence[61] and immunoautoradiography have demonstrated AFP in the cytoplasm of hepatocytes and hepatomas, but no incorporation of

radiolabeled amino acids into AFP has been demonstrated in the spleen, kidney, lung, placenta, intestine, stomach, heart, or brain.[22, 61–67] Van Furth and Adinolfi,[68] however, obtained results suggesting AFP in two of seven placentas. Clarification of this point would be clinically useful in obstetrics. In cases of molar disease, elevated levels of HCG but not of AFP have been reported.[69].

Although dispute still exists as to the origin of AFP from hematopoietic cells of liver and blood,[70] most investigators agree that AFP content varies from cell to cell and that AFP-producing cells commonly form islands around hepatic blood vessels.[71] Incorporation of amino acids into AFP by cells in culture has been demonstrated by Gitlin and Boesman,[65] and Van Furth and Adinolfi.[68] Evidence for other specific cells of origin for AFP will be cited under the section dealing with induced hepatomas. Discussions on the dynamics of AFP production during embryonic and fetal life are provided by Andreoli and Robbins,[8] de Muralt and Roulet,[7] Tatarinov,[9, 72] Masopust and Kotel,[10] Gitlin and Boesman,[11] and Van Furth and Adinolfi.[68]

Sources for purification. AFP has been detected in fetal and maternal serum,[19] fetal urine,[11, 47, 66] amniotic fluid, and cerebrospinal fluid.[47] Of these, Masseyeff[19] considered human fetal blood to be the best source of AFP for purification, since at 20 weeks a concentration of 20 mg. per milliliter is found. Cord blood and ascitic fluid were regarded as secondary sources because of the lesser amounts of AFP available per specimen. In the literature, pooled cord blood is the chief source of AFP for purification.

Purification of AFP. One reason AFP may have eluded detection for so long is its close assocation with the albumin fraction on zone electrophoresis (Fig. 1). Separation from albumin has been a problem because their biochemical and physicochemical features are so similar. Another problem is the much higher concentration of albumin (40 mg. per milliliter) than AFP (2 mg. per milliliter). Biochemical separation is difficult because of the biochemical similarities between the two substances,[19] yet, Rapp and Lehmann[73] and Alpert and associates[55] achieved a fair degree of purification by biochemical methods using ascitic fluid and fetal serum. Mixed biochemical and immunochemical methods were used by Purves and associates,[74] Goussev and Jasova,[75] and Lehman and associates.[76] Abelev[15] regarded the Goussev and Jasova method to be the simplest and most effective means of purifying AFP. These investigators also obtained a very specific antibody.[77] Immunochemical methods of purification have been reported by Nishi and Endo[51, 78–80] and their co-workers, Ruoslahti and Seppälä,[53] and Page.[81] The Nishi preparation has been widely distributed and mentioned in recent reports.

Antibody production and purification. Antigens have included normal fetal serum and serum from patients with either hepatoma or cirrhosis.[82] An advantage of using semipurified or purified AFP rather than whole serum fractions is the avoidance of "antigenic competition."[54, 55, 83] The use of minute amounts of antigen is provided by the popliteal node route of injection.[83, 84] Absorption with healthy adult serum has been used to obtain a more specific antibody preparation.[85, 86]

Assay methods. Gel diffusion which has a sensitivity of 1 to 3 μg per milliliter has been reported by several investigators.[15, 19, 86, 87, 88] Lacking a high degree of sensitivity, this method failed to pick up lower levels of AFP in patients with hepatoma and led to the erroneous conclusion that AFP did not appear in the serum of normal or pregnant adults.

The radioimmunodiffusion method of Mancini and associates [89] has been used by Nishi for semiquantification of AFP.

The Laurell rocket technique[90, 91] is a one-dimensional antigen-antibody crossed electrophoresis which gives a quantitative estimate of protein. It has been used by Seller and associates[92] for antenatal diagnosis of anencephaly by screening amniotic fluid for AFP.

Counterimmunoelectrophoresis (CIEP) has also been known as electroimmunodiffusion and immunoosmophoresis. It is approximately 40 times more sensitive than the Ouchterlony method[19] and has been described by Alpert and associates.[93] It is currently being used as a screening test in a prospective study on every patient in the prenatal clinic at the Johns Hopkins Hospital. A sensitivity of 62.5 ng. per milliliter has been achieved, although a sensitivity of 125 or 250 ng. per milliliter would be clinically acceptable, since many abnormalities in obstetrics seems to be associated with levels above these.[94]

Rivanol counterelectrophoresis[95] has been described as a method preferable to radioimmunoassay for the serologic screening of primary liver cell carcinoma. Rivanol is used to concentrate the AFP by five- to ten-fold; a higher degree of specificity was reported for this method than for radioimmunoassay (RIA), which did not increase the rate of detection.

Aggregate hemagglutination (AH) was used prior to RIA as a highly sensitive test. Reportedly 10,000 times more sensitive than an immunoprecipitin reaction, it has a sensitivity of 50 ng. per milliliter.[15, 96, 97] A sensitive hemagglutination test has been reported by

Hirai and Nishi.[98] Complement fixation has been used by Kahan and Levine[99] for mouse AFP.

Radioautography[15, 97, 100, 101] has been reported to be 15 to 30 times more sensitive than the Ouchterlony method and "absolutely specific."[15]

An immunoenzymatic double-antibody assay for serum AFP, using horseradish peroxidase conjugated to sheep anti–rabbit IgG with a sensitivity of 50 ng. per milliliter, has been reported by Alpert and Coston.[102]

A latex slide test (LS) described by Alpert and Coston[103] is another of the screening tests currently used in the prospective study at the Johns Hopkins Hospital. Its reported sensitivity is 250 ng. per milliliter, which is acceptable for screening clinical abnormalities in pregnancy and for diagnosis of hepatocellular carcinoma.[104]

A variety of RIA's have been reported by several investigators.[84, 105–115] One would think that the ultimate test had been reached with a method giving a high degree of sensitivity like the radioimmunoassay. Such, however, is not the case, as wide differences in results are noted in the same sample when dilutions are made on specimens containing a high concentration of AFP. This has led Mukojima and associates[116] to recommend that a less sensitive method like simple radial immunodiffusion be combined with a radioimmunoassay to "titrate the whole range of AFP distribution." This combination, they contend, will take care of both the wide distribution of AFP in various diseases and the dilution error frequently encountered in RIA.

The choice of a suitable test or combination of tests for various clinical conditions remains to be determined. The problem may be likened to the initial screening for Rh disease, where an Rh titer was not found to be necessary for each and every pregnant woman. Instead, a simple typing for Rh now constitutes the preliminary screening process and the titrating is reserved for women who are Rh negative. A prospective study is now under way at the Johns Hopkins Hospital to compare the clinical usefulness of so-called "simplified assays" for AFP (CIEP, AH, and a latex slide test) with a more complicated double-antibody radioimmunoassay. The current impression is similar to that of Mukojima—that both types of tests have their distinct advantages for prenatal screening.

Standardization. In comparing normal values for AFP in amniotic fluid, Seppälä and Ruoslahti[117] noted a twofold difference between their results and those of Brock and Sutcliffe.[118] They postulated that the use of an impure or contaminated standard may in part cause such differences.

In our experience, the fetal cord serum used as our RIA standard was assayed by Drs. McIntire and Waldmann at the National Institutes of Health by the Laurell method and RIA. The range of results was from 75,000 to 117,000 ng. per milliliter, with the use of dilutions causing problems in the RIA.

The adoption of the WHO standard 72/225 as proposed by Sizaret[119] would aid in eliminating such differences due to use of local standards. The 72/225 standard prepared from cord sera has been assayed by nine different laboratories with a range of AFP values from 27 to 90.5 μg per milliliter. The preparation has a half-life of 162 years at −20° C. and various aliquots are homogeneous.[119] The use of this standard may give results which are comparable between laboratories using different methods.

Physiology and immunology.

Possible functional roles of AFP. Although the function of AFP is unknown, recent work has strongly suggested possible roles.

Uriel and associates[120, 121] have studied the estrogen-binding properties of AFP. Using cell affinity labeling with tritiated estrogens, they found that the transitional cells (the AFP-producing cells) were labeled the most. The reaction appears to be specific, as no binding could be demonstrated for testosterone, progesterone, cortisone, or dexamethasone. The possible functional significance of this is expressed in the Leffert theory, as reported by Sell and associates[122]: Intracellular AFP by binding intracellular estrogen may inhibit low-density lipoproteins which are ordinarily stimulated by estrogens and which usually inhibit AFP. Estrogen binding has also been studied by Nunez and associates[123] in the rat and by Nishi and Hirai[52] in human subjects. The interrelationships of AFP with HCG (human chorionic gonadotropin) and steroids are only beginning to be considered.[124] Its role as a growth-stimulating factor has been proposed[125] and questioned.[134]

A possible role of AFP in suppressing the immune response has been studied by Murgita and Tomasi.[126, 127] AFP may have an immunoregulating function which is important in protecting the fetus from immunologic attack by the mother's antibodies or in protecting a tumor from attack by the host's antibodies. In one series of experiments,[128] both mouse amniotic fluid containing AFP and isolated AFP acted as noncytotoxic inhibitors of the primary-cellular synthesis of γM antibody in splenic plague-forming cells to sheep red blood cells and the secondary response of γM, γA, and γG synthesis. In a second series of experiments,[127] AFP was shown to suppress thymus-derived T cell–dependent functions of allogenic and mitogen-induced lymphocyte transformation. A proposed mechanism for the immunosuppression by AFP is that the helper

T cells for antibody synthesis may be inhibited. The reactions are time dependent and may require macrophages or B (bone marrow) cells for effective suppression of antibody production. Thus, elevated levels of AFP could depress the maternal immune response and delay the rejection of a histoincompatible fetus and could explain the reduced capacity of the fetus and newborn infant to respond to antigenic stimuli. The lifting of immunosuppression after birth may be correlated to the disappearance of AFP. The findings of these investigators may also explain the appearance of high levels of AFP and concomitant immunosuppression reported in liver disease and some tumors.

Waldmann and McIntire[109, 128] reported elevated levels of AFP above 30 ng. per milliliter (range 44 to 2,800 ng. per milliliter) in patients with ataxia-telangiectasia (AT). This disease is characterized by an immunodeficient state, the absence of a gamma globulin, recurrent infections, telangiectasias of the conjunctiva in skin, cerebellar ataxia, apraxia of ocular movement, and choreoathetosis. Elevated levels of AFP were detected in 54 other patients with other immunodeficient states or in parents and siblings of patients with AT. Here, immunosuppression at the improper time may have a detrimental role.

Model for cancer studies. Seppälä,[47] Seppälä and Ruoslahti,[129, 130] and Cohen and associates[94] have shown the absence of maternal antibodies to fetal AFP. Why does a mother not produce antibodies against fetal AFP? The search for an answer uses the AFP–anti-AFP system as a model for the study of specific therapy for cancer and as a model for the study of maternal-fetal immune mechanisms.

Ruoslahti and Pihko[131] studied the breakdown of tolerance to AFP in rabbits and mice. Japanese investigators, according to Ruoslahti, had previously showed that antibodies against AFP, which do not appear naturally, can kill AFP-producing cells in the liver. Rabbits immunized with rabbit AFP did not produce antibodies to AFP, but rabbits immunized with a slightly modified rabbit AFP (haptenated AFP) formed antibodies against homologous AFP. Both immunocompetent cells, the thymus-dependent (T) cells and the bone marrow (B) cells, are needed for the formation of humoral antibody according to Ruoslahti. The theory is that the B cell recognizes a portion of the antigenic site on the AFP molecule, but cannot induce antibody synthesis until a haptenic group provides the AFP molecule with a portion recognizable by the T cell receptor. Ruoslahti believes that the small but persistent levels of AFP in the nonpregnant adult state may be responsible for tolerance in the pregnant state.[131]

Nishi and Hirai[132] reported two significant experiments which suggest a specific cancer chemotherapy. They confirmed that extensive immunizations of a rabbit with rabbit AFP failed to produce antibody. The breakdown of immune tolerance to homologous AFP in the rabbit was achieved by injection of human AFP. In the first experiment, seven rabbits were given human AFP and produced antibody to the homologous AFP. Then pregnancy was achieved in five rabbits, all of whom gave birth at the usual time. Although there was no apparent abnormality in the neonates, severe damage of liver cells was found in microscopic sections of the fetal liver, but no damage was found in fetal spleen or kidney. Antibody to rabbit AFP in maternal serum was high in early pregnancy and decreased with gestation. In a second experiment, similar liver damage was noted in a newborn rate of a mother injected with horse anti–rat AFP. The significant findings in both experiments are that maternal antibody was transferred to the fetus where it exerted damage and that the damage was specific to AFP-producing cells.

This cytotoxic effect described by Nishi and Hirai differs from the noncytotoxic effect of AFP described by Murgita and Tomasi.[126] A mechanism of the cytotoxic effect, which is of importance for a specific cancer therapy, was proposed by Tsukada and associates.[133] They found horse anti-AFP adhering to AFP secreted on the cell surface and postulated that a complex deposited at this site might inhibit cell multiplication in a manner analogous to that proposed for CEA (carcinoembryonic antigen). CEA had been found on the surface of tumor or embryo cells, not as an integral part of the cell membrane, but as extracellular material forming a glycocalyx. AFP might also be forming a glycocalyx.

Diseases of the liver

Production and release of AFP. It is important for the obstetrician to be acquainted with AFP and the liver. Not only will the knowledge help assess fetal growth, but it will also help in understanding AFP levels in abnormal states in pregnancy. Recently a case report[124] described a hepatocellular cancer in a pregnant woman in whom the diagnosis was missed in spite of a concentration in maternal serum of 25 μg per milliliter (note the use of micrograms instead of nanograms).

Experimental situation. Studies of AFP production in the liver take advantage of naturally occurring hepatomas, those induced by chemical agents,[15] or the rapid regeneration of hepatocytes caused by either surgical or chemical hepatectomy (CCl_4).

Factors in naturally occurring tumors. Naturally occurring hepatomas, commonly referred to as PLC (primary liver cancer), are influenced by many factors such as geographical location, age, and sex. PLC is relatively infrequent in the United States of America, England, France, Greece, and the U.S.S.R. It occurs in high frequency in the Asian countries and Senegal, the Union of South Africa, Mozambique, Kenya, Uganda, and the Congo.[15, 19] Young affected patients have much higher levels of AFP than older affected patients, with no substantial decrease in the difference until after age 40, when a 20 to 40 per cent decrease occurs.[19] Other reports confirm this age factor.[93, 135, 137] This probably explains why 71 per cent of the patients with PLC from the Senegal with an average age of 38 years have elevated AFP levels whereas only 53 per cent of patients from France with an average age of 63 years have elevated levels. Sex is a factor in that more males are positive for AFP than females.[93, 135, 138] In nontropical countries the male to female ratio is 1:1 to 4:1; in Africa and Asia the ratio is 5:1 to 10:1. In these patients, AFP has been measured in ascitic fluid,[139] CSF, saliva, urine, and blood.

Factors governing appearance and level of AFP. AFP is not found in all liver tumors or liver diseases. The rate of positivity of AFP varied according to age and geographical location, as has been stated, but the problem is more complex than this. Abelev[15] stated that abnormal AFP levels were found in only 78 per cent of histologically confirmed cases of hepatocellular cancer. This means that 30 per cent would have been missed by AFP screening. Alpert and Coston,[102] using a sensitive method (50 ng. per milliliter), obtained positive AFP results in 75 per cent of hepatoma cases, 13 per cent of acute viral hepatitis cases, 44 per cent of massive hepatic necrosis cases, and 23 per cent of chronic active hepatitis cases. Negative or low AFP results were obtained for other gastrointestinal tumors, acute or chronic nonviral liver disease, and posthepatic lobectomy in a rapidly regenerating phase. Abelev suggested that in nonmalignant disease the AFP elevation may be due not to hepatic regeneration but to viral injury. The sensitivity of assays employed may influence the yield of cases with positive AFP results as shown by O'Connor and associates,[138] who showed that, in the same series of cases, 63 per cent were positive for AFP by immunodiffusion but 85 per cent were positive by radioimmunoassay. The range of AFP concentrations is great—from 0.02 to 1,000 mg. per 100 ml.[93] It is probably easier to remember that in PLC levels go from the nanogram to the milligram per milliliter range.

Certain pathologic features are correlated with the appearance of AFP in liver tumors. Size of the tumor was related to the number of samples positive for AFP,[135, 140] but not to the level of AFP.[93, 106, 135] A correlation of AFP levels with microscopic features showed that anaplastic lesions had the lowest levels (16 mg. per 100 ml.), well-differentiated lesions had intermediate levels (25 mg. per 100 ml.), and poorly differentiated lesions had the highest AFP levels (32 mg. per 100 ml.)[19]

Only a small porportion of tumor cells make AFP.[19] Less than 5 to 20 per cent of cells have been found by immunofluorescence to be positive for AFP. These cells occur in characteristic sleeves around capillary or tumor sinuses. Cells farther away from vessels were negative for AFP. More recently, Onoé and associates[141] demonstrated a drastic fluctuation of cell populations and the appearance of AFP. Degeneration of hepatocytes was followed by a proliferation of cholangiolar oval cells which function as blast cells of hepatocytes in the early stage of regeneration. They believed that the oval cell retraces the process of ontogenetic differentiation to a transitional cell which is intermediate between a typical oval cell and hepatocyte. These transitional cells are the ones which produce AFP as suggested by both immunofluorescent studies and the appearance of AFP. Besides cell type, other factors regulate the fate of AFP.

Factors regulating synthesis and release. The production and release of AFP are governed by several factors, as recently reported. Sell and associates[122] found two patterns of release of liver-specific proteins in monolayer cultures of fetal rat hepatocytes. Kanai and associates[142] found AFP synthesized mainly on membrane-bound polysomes in rat ascites hepatoma cells as shown by the incorporation of radioactive labeled leucine. Cyclic AMP was found to increase the production of AFP in Yoshida sarcoma cells.[143] Production of AFP in the cytoplasm of rat hepatoma cells (mainly in the rough endoplasmic reticulum) was demonstrated by immunoelectron microscopy with peroxidase-labeled anti-rat AFP.[144] No involvement of the Golgi apparatus was found. Pyridoxine deprivation was found to induce the appearance of AFP and liver nodules in five of 10 baboons.[145–147]

Cells can produce proteins only at certain stages of their mitotic cycle. Sell and associates[122] found that only 20 to 30 per cent of cells divide when stimulated and that only dividing cells make alpha$_1$-F. Synthesis occurred in the G_1 phase with release in the G_2 phase. Translated, this means that AFP appears after the initiation of cell proliferation, increases linearly in amount during the active phase of growth, and reaches a plateau when the highest number of cells are found.

An intracellular half-life of 1 hour was found for AFP and 6 hours for albumin, which means that the synthesis and release of AFP occurred more rapidly than of albumin. Production of AFP was not blocked by the inhibition of DNA synthesis, but was blocked by the inhibition of mitosis. Tsukada and Hirai[148] also studied AFP synthesis and cycles of cell division and noted that almost all cells produce both AFP and albumin, with some cells producing more AFP than albumin. Thus, factors controlling the regulation of AFP production which explain the appearance of AFP in various clinical states are slowly evolving.

Theories for reappearance of AFP. Various hypotheses for the reappearance of AFP in cancer, both of the liver and other tumors, have been proposed. That cancer cells resemble embryonic cells and vice versa, has been stated many times. In 1932, Hirszfeld and associates[149] were the first to assert the existence of fetospecific substances in cancer tissue.[19] In 1963, Abelev and associates[150] issued the first report showing this association.[19] Abelev[15] has proposed three theories: a cell multiplication theory, a cell dedifferentiation or retrodifferentiation theory, and a stem-cell theory. These and other theories are discussed by Masseyeff,[19] Abelev,[85] Irlin and associates,[151] and Uriel.[22]

ASSOCIATION WITH HEPATITIS B ANTIGEN. An unusually high incidence of Australian antigen (hepatitis B antigen) has been found in association with hepatoma.[31, 152–156] An impaired immune mechanism or an immunodeficient response to hepatitis B antigen, possibly on a genetic basis, has been proposed and studied by Sakurai and Miyaji[153] of Osaka and Simons and associates[154] of Singapore. In Osaka, 30 per cent of 72 cases of hepatoma had the hepatitis B antigen, whereas only 7 per cent had the antibody to hepatitis B antigen. In screening for both Australian antigen and AFP in the Japanese town of Tomi, 9.9 per cent of the population was positive for the antigen in contrast to 1.5 per cent, which is the average figure for Japan. All cases with positive antigen had elevated levels of AFP.[156] A combination of these two survey methods may improve the early detection of hepatoma in countries having a high incidence of this disease.

SERUM AND INFECTIOUS HEPATITIS AND CIRRHOSIS. In serum or infectious hepatitis, elevated levels of AFP have been found in a fraction of the cases. Abelev[15] reported 13 per cent of patients with hepatitis had AFP levels in the range of 50 to 500 ng. per milliliter, which disappeared after convalescence. The total period of appearance and disappearance was less than 2 months. Silver and associates[157] found approximately 3 per cent of 128 adult patients had elevated AFP levels which seemed to be correlated to the severity of liver destruction, as indicated by serum glutamic pyruvic transaminase.

Cirrhosis is considered by some[158] to be a precancerous disease for PLC. In Japan, more cases of liver cirrhosis (53.8 per cent) showed elevated levels of AFP than cases of chronic hepatitis (18.7 per cent).[155] AFP levels in cirrhosis exceed 100 ng. per milliliter, with levels above 400 ng. per milliliter strongly suggesting the presence of a hepatocellular cancer. Lehmann[158] used AFP levels to follow the malignant transformation of liver cirrhosis to primary liver cancer. The presence of hepatitis B antigen in all cases of cirrhosis with high levels of AFP has been reported by Endo and associates.[155]

Other tumors and diseases

Other tumors. Day[159] has chosen to call the AFP assay cancer distinctive rather than cancer specific. This is because false-positive results may be obtained in cases of hepatitis and cirrhosis as noted. Another reason is that AFP levels may be elevated in tumors other than those derived from the liver.

Abnormal levels of AFP would be expected in tumors containing elements of embryonal carcinoma. Abelev[15] found positive AFP values in 50 per cent of his cases. Other embryonal tumors should have no AFP: seminoma, chorionepithelioma, neuroblastoma, nephroblastoma, and Wilms' tumor. It was postulated that AFP assays would have clinical value in the differential diagnosis of germ-cell tumors and in predicting the efficacy of surgery and chemotherapy. The actual experience is as follows. AFP has been found in hepatoblastoma (different from hepatocellular carcinoma in being embryonal)[160–162] and a large group of teratoblastomas containing solid undifferentiated tissue without organized structure. Teratoblastoma in the testis with elevated AFP levels has been reported by Hull[163] and Mawas[164] and their colleagues. Abelev[86] found AFP in 10 of 26 embryonal tumors and one in 18 seminoma or chorionepithelioma of the impure type. Positive AFP levels have been reported in teratoblastoma in the ovary,[164–166] the retroperitoneum,[164, 165] the mediastinum,[164] and in sacrococcygeal tumors.[164] More recently, Mukojima and associates[167] have reported the presence of AFP in 9.5 per cent of cases with metastatic liver cancer and 54 per cent of cases with embryonal cell carcinoma by the single radial immunodiffusion method. Tsuchida and Ishida[168] reported seven cases of teratocarcinoma that were AFP positive: three in the sacrococcyx, two in the ovary, and two in the testis, five of which showed typical features of a yolk sac (endodermal sinus) tumor. They contended that these tumors are of yolk sac

origin. No AFP has been found in a pure cholangiocellular carcinoma,[15] nephroblastoma, or sympathicoblastoma.[165] The specificity of a particular laboratory method has been questioned in a study[97] which showed the presence of AFP in seminoma, chorionepithelioma, nephroblastoma, malignant blood diseases, and metastatic liver disease.

Stomach. Tumors of the stomach and abnormal AFP results have been reported by several workers.[135, 140, 169–172] Mukojima and associates[167] found AFP in 17 per cent of cases with gastric cancer with hepatic metastatis but in only 0.6 per cent of cases without hepatic metastasis using a single radial immunodiffusion technique. With a radioimmunoassay, the figures increased to 71.4 per cent and 39.5 per cent, respectively.

Colon, pancreas, prostate. Elevated AFP levels associated with primary tumors of the colon have been reported by Bernades[173] and Andrieu[174] and their co-workers. Primary tumors of the pancreas[135, 140] and prostate,[172, 175] usually in association with hepatic metastasis, have also been reported. Of interest is the presence of gastric tumors (one definitely primary) in both cases of prostatic adenocarcinoma.

Other diseases. The presence of AFP in ataxia-telangiectasia, an immunodeficient disease, has been mentioned previously.

For the sake of completeness, it should be mentioned that elevated levels were reported in 11 of 11 patients with acute or subacute hereditary tyrosinemia[176] and in one of six cases of infectious mononucleosis,[177] conditions which may be related to regenerating liver tissues as in cirrhosis and viral hepatitis. Because one might anticipate levels of serum AFP in blood disorders associated with myelopoiesis, Masseyeff and associates[178] screened 50 cases of various blood dyscrasias and found no raised levels of AFP in acute and chronic myeloid leukemia, acute lymphoid leukemia, chronic lymphocytic leukemia, lymphosarcoma, chronic myeloid leukemia, reticulosarcoma, myelomatosis, Hodgkin's disease, Waldenström's disease, Biermer's disease, hemoglobinosis C, thymoma, osteomyelosclerosis, and idiopathic bone marrow aplasia.

AFP in pregnancy

Introduction.

Discovery. When Tatarinov in a personal communication reported to Masseyeff[19] that he detected AFP in six of 1,009 pregnancies, it seemed like a monumental discovery. In the late 1960's, others[11, 135, 138, 179] reported an inability to detect AFP in pregnant women. In 1970, Foy and associates[71, 147] and others reported a higher incidence of AFP in pregnancy and it soon became apparent that insensitive methods like gel diffusion were not picking up the nanogram quantities of AFP present in maternal serum. With the development of radioimmunoassays, Abelev,[13, 180] Purves, Geddes[181, 182] and Seppälä and Ruoslahti[130] were all able to find levels of AFP above the nonpregnant adult level of 10 ng. per milliliter. Ishiguro and Nishimura[106] detected AFP as early as 14 weeks and noted a maximum level of 386 ng. per milliliter at 36 weeks of gestation. By means of a radioimmunoassay, AFP was detected in the serum of a patient with hydatidiform mole and in the urine of a pregnant patient with marked albuminuria. Ishiguro[183] also noted that no difference in level was observed between primiparous and multiparous patients.

Scope of uses. This discussion considers the indications for measuring AFP as they might arise in following a patient throughout the course of pregnancy. AFP testing in maternal serum, amniotic fluid, and fetal serum will be considered simultaneously. This is in preference to the approach which considers individually first the disease entities which can be detected by maternal serum, then those which can be detected by amniotic fluid, and finally those which can be indicated by fetal serum. It is hoped that the scheme presented will be more useful to the physician when ordering AFP assays for a given case.

There are a few important facts that must be remembered when considering AFP in pregnancy. The concentration gradient ranges from milligrams per milliliter in the fetus to micrograms per milliliter in amniotic fluid to nanograms per milliliter in maternal serum. Fetal serum concentration must be distinguished from total amount of AFP produced in the fetus. Fetal serum concentration declines from a peak of 3,000,000 ng. per milliliter at 14 weeks to less than 13,000 to 86,000 ng. per milliliter at term. By contrast, the total AFP synthesized reaches a peak at 20 weeks, remains constant from 22 to 32 weeks, then drops steeply to birth, after which it tapers off. Maternal levels at the upper limits of normal can be taken as 125 ng. per milliliter in the first trimester, 250 ng. per milliliter in the second, and 500 ng. per milliliter in the third trimester. Seppälä and Ruoslahti[184] found levels of 18 to *119*, 96 to *302*, and 103 to *550* for each of the trimesters, respectively. Because of the difference in standard preparations used, these values may have only approximate value in other laboratories. For convenience, the values of 125, 250, and 500 ng. per milliliter are used, which are easier to remember than 119, 302, and 550 ng. per milliliter. On a log scale (Fig. 2), the values are essentially identical for clinical purposes. The AFP in maternal serum is directly contributed by the fetus, as shown in a definitive

experiment by Seller and associates[186] in which labeled AFP was traced from the fetus into maternal serum. Single assays are probably not as valuable as serial titrations, especially since standard preparations and levels vary. For this reason, the slope of the line between values should also be observed to obtain useful clinical information. Amniotic fluid levels, which reflect fetal levels, decline from 30,000 ng. per milliliter in early pregnancy to less than 50 ng. per milliliter at term. In searching for an explanation of an abnormal AFP level (high or low), one should consider alterations in fetal liver production, the volume of body fluids involved, changes in plasma permeability, and disturbances in the circulation and elimination of amniotic fluid, and not solely fetal-maternal transfusion. With a half-life of 3 to 4 days, AFP is cleared to negligible levels in maternal serum by 20 days post partum.[108]

Uses in pregnancy. The uses of AFP in pregnancy can be categorized according to indications in early pregnancy and later pregnancy (Table I). The indications for use of AFP in pregnancy change throughout all three trimesters. This is in contrast to most other biochemical fetal monitors, which are useful in only one period of pregnancy. In the first trimester, AFP assays may be used to predict outcome in threatened abortion, to select cases of cervical incompetence for suture, and to screen for early birth defects. In the second trimester, the indications for AFP change to screening for fetal distress, assessing the severity of diabetes, Rh immunization, toxemia, and screening for the development of later birth defects. In the third trimester, some of the indications for AFP assays include the determination of gestational age, the detection of fetal distress, the prediction of fetal death, and the evaluation of innocuous versus serious bleeding. These indications and others will be considered in detail.

Various conditions.

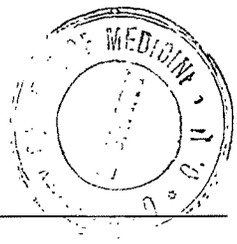
Threatened abortion. The prediction of outcome in threatened abortion can result in considerable saving in days hospitalized, in maternal blood loss, and in emotional stress by preparation for the predicted outcome.

Prediction of the outcome of threatened abortion was first reported by Seppälä and Ruoslahti,[187] who found that after the thirteenth week of gestation abortion occurred more frequently in women with abnormally elevated AFP results. A case of blighted ovum with low levels and cases of intrauterine fetal death with high levels of AFP were reported. A more recent paper[48] combines the use of human placental lactogen (HPL) with AFP screening to improve predictive ability. The difference is that HPL reflects placental status whereas AFP reflects fetal status, and the two complement each other in predicting over-all status of the pregnancy. Seppälä's category of normal AFP levels included the abnormally low levels below the 2.5 percentile, which may be reflective of diminished or absent fetal liver production.

Table I. Reported indications for AFP assays

Early pregnancy:
- Predict outcome of threatened abortion
- Signal a blighted ovum vs. growing fetus
- Select cases of incompetent cervix for surgery
- Differentiate between mole and normal pregnancy

Later pregnancy:
- Detect fetal distress
- Predict impending fetal death
- Monitor maternal complications, e.g., toxemia, diabetes, Rh immunization
- Screen for twins
- Detect neural tube defects
- Monitor for other birth defects
- Assess uterine bleeding
- Predict (unexpected) stillbirth
- Detect cancer in pregnancy
- Assess gestational age
- Other



Spontaneous vs. induced abortions. Because early intrauterine deaths in both spontaneous and induced abortions are probably the result of either increased fetal-maternal transfusion or resorption of fetal elements, elevated levels are expected in both maternal and amniotic fluid. Seller and associates[189] found elevated levels ranging from 50 to 305 μg per milliliter of amniotic fluid in 54 cases of spontaneous abortion with intact sacs. The affected fetuses had severe neural tube defects or chromosome abnormalities such as 45, X. Unaffected fetuses had AFP levels below 50 μg per milliliter. AFP levels increased in maternal sera from the preabortal to the postabortal states.[187]

Cervical cerclage. Cervical cerclage in women with elevated serum AFP levels resulted in abortions.[187] Identification of fetuses who are doomed to abort may reduce needless surgery in these cases. Seppälä[47] stated that maternal defects can be distinguished from fetal defects.

Choriocarcinoma and mole vs. pregnancy. The differentiation of choriocarcinoma or hydatidiform mole from normal pregnancy can be helped by AFP assays if one assumes that the serum level will not be elevated in the absence of a fetus. This was substantiated in one report,[69] but further study is necessary to explain the appearance of AFP in the serum of one patient with hydatidiform mole.[108] The possibility of the presence of a fetus with mole has to be considered.

Fetal death. Fetal death later in pregnancy is alsc attended by increased fetal-maternal transfusion and

elevated levels of AFP in both maternal serum and amniotic fluid. Because the increase in maternal AFP occurs before fetal death, elevated or rising levels of AFP can be used to detect serious fetal distress and impending death.[188] Thus, the stress of severe toxemia which led to a compromised but living newborn infant in one case but to fetal death in six other cases was reported by Cohen and associates.[94] In high-risk pregnancies[188] 60 per cent of cases with fetal distress were correctly predicted. The severity of fetal distress and a probability of fetal death increased as levels increased from 800 to over 1,000 ng. per milliliter. Twin pregnancies and high AFP levels with the death of one fetus have been encountered by different authors.[94, 188] Further work is necessary to define the types of fetal distress which may be indicated by AFP levels.

Toxemia. After the first trimester, AFP assays may be useful in assessing maternal complications. In toxemia, Rh immunization, and diabetes, patterns in the level of AFP change in the same direction in fetal serum, maternal serum, and amniotic fluid. In toxemia, 65 per cent of cases fall below the median level in maternal serum and are also below median levels in fetal serum and amniotic fluid. This is possibly related to either a small placenta, or an acceleration of maturation due to stress, or both.[47] Abnormally high levels might be associated with severe fetal distress, as occurred in an infant of 1,430 grams with an Apgar score of 6 at 37 weeks.[94]

Diabetes. In diabetes, 65 per cent of cases have AFP levels above the median in maternal serum and also in fetal serum.

Multiple gestation. Ishiguro[189] found AFP levels twice as high as normal levels. As many as 98 per cent of cases may have AFP levels above the median level, with fetal death being signaled by levels exceeding the 97.5 percentile.[47, 49] Garoff and Seppälä[49] found that elevated levels of AFP and HPL were useful in detecting and predicting the fate of twin pregnancies.

Rh immunization. Rh immunization has levels elevated above the median in all three compartments. In Rh immunization, a large placenta and activation of hematopoiesis may explain the increased levels. In maternal serum, 72 per cent of cases were found to have AFP levels above the median.[47] Maternal serum levels did not seem to predict intrauterine death in Rh disease for Vivian and Ward.[190] On the other hand, because AFP levels rose before fetal death, they were judged to be useful in predicting outcome in maternal serum by Cohen and associates[94] and in amniotic fluid by other investigators.[96, 191, 192]

Innocent vs. serious bleeding. A distinction between innocent and serious bleeding in pregnancy may be indicated by AFP levels, according to an early report.[193] High levels associated with an increased fetal-maternal transfusion would indicate a serious prognosis, but further study is needed to correlate different levels of AFP with different types of antenatal hemorrhage, such as placenta previa, abruptio placentae, and marginal sinus bleeding.

Birth defects. Down's syndrome[194] and ataxia-telangiectasia are conditions associated with elevated AFP levels which can be recognized at or after birth. Other conditions include congenital nephrosis, tetralogy of Fallot, duodenal atresia, and esophageal atresia. In the latter, the mechanisms of elevated AFP levels in amniotic fluid either may be by inhibited fetal swallowing or by regurgitation of gastric contents through a fistula—or by both.[47] The prenatal detection of these conditions may help to improve both the emotional care of the parent and the medical care of the infant.

Certain chromosomal abnormalities such as 45, X may be associated with elevated amniotic AFP levels.[186] Normal AFP levels in amniotic fluid have been found in trisomy G-21. Further studies are necessary to elucidate the mechanisms involved in AFP synthesis with various chromosomal patterns.

Gestational age estimate. Gestational age dating is a real problem for the obstetrician. Seppälä[47] stated that 15 per cent of English women do not know their LMP. The dating of gestational age has been attempted in fetal and newborn serum[195, 196] and amniotic fluid.[197] In both, a high correlation has been found between AFP levels and gestational age but not between AFP levels and fetal weight. Determinations in amniotic fluid seem to provide a finer index of gestational age in late pregnancy than early pregnancy, since the scatter of values is greater in earlier pregnancy.

The coefficient of correlation between alpha-fetoprotein and gestational age in fetal serum was found to be higher than the correlation between birth weight and gestional age, especially from 33 to 47 weeks by Karlsson and associates.[198]

Seppälä[47] has stated that AFP levels in amniotic fluid below 70 ng. per milliliter indicate a pregnancy of at least 34 weeks whereas levels below 50 ng. per milliliter are indicative of pregnancies of 37 or more weeks of gestation.

Neural tube defects.

Definition and classification. Defining the type of neural tube defect (NTD) is important because the type of lesion will help indicate the levels of AFP that may be expected and vice versa. Open tube defects include anencephaly, myelocele, cervical meningocele, lumbar myelocele, hydrocephalus, and myelocele with hy-

drocephalus.[199] These open lesions are associated with unrestricted leakage of AFP into amniotic fluid and thus into the maternal compartment. Closed lesions not only include those with a full-thickness skin cover, but are considered by some to include small "open" lesions with restricted leakage of AFP into the amniotic fluid and maternal compartments. Harris and associates[200] included encephaloceles, a few myeloceles with full-thickness skin covers, uncomplicated hydrocephalus, and small "open" myeloceles and meningoceles under the category of closed lesions. Laurence and associates[201] cited that 14 per cent of infants with neural tube defect who survive have closed lesions. Of these, 6 per cent are encephaloceles with severe brain abnormalities, 4 per cent are meningoceles with cord involvement, and 4 per cent are closed myeloceles or severe neural defects with thick epithelial coverings. By contrast, a figure of only 4 per cent is usually cited as the number of neural tube defects compatible with survival by another source.[202] Of various types of spina bifida, myelomeningoceles and myeloceles (containing meninges, spinal fluid, spinal cord, and nerves) are common, severe, and crippling by comparison to meningoceles (containing meninges, spinal fluid, but not spinal cord or nerves) which are less common and benign. Warkany[203] discussed these defects and hydrocephalus, uncomplicated hydrocephalus, encephalocele, iniencephaly, and exencephaly in an excellent review of congenital malformations. Freeman's[204] book *Practical Management of Meningomyelocele, A Multidisciplinary View* further discusses the classification of "open" and "closed" lesions.

Risks involved. The risk involved of having another child with NTD is of interest in genetic counseling. Most of the studies related to NTD have been reported from South Wales and Belfast, where the incidence is high. But the risk of study techniques must be balanced against risk of the disease. Because the risk in amniocentesis as reported by Scrimgeour[205] was approximately 1 per cent, amniocentesis in those studies was justified only when the risk of a fetal malformation was greater than 1 per cent. As shown by Carter and Roberts[206] and Laurence,[207] the risk of having another child with NTD is 1/20 to 1/25 after one previously affected child and 1/10 after two previously affected children. The risk of having an affected child from an adult survivor of either sex with spina bifida is 3 per cent.[208] If an affected child is the index case, the risk of spina bifida in the mothers' sisters' children is 3 in 266 as reported in a study covering six areas in England and Wales where the random incidence was 2.5 in 1,000 total births.[206] With the techniques described,[206] the risk of a living child with serious neural tube defects can be reduced from 1 in 10 to less than 1 in 100.[200]

Factors involved. Since Brock and Sutcliffe[118] first reported the value of AFP in amniotic fluid in the antenatal diagnosis of neural tube defects, an avalanche of studies has clarified the importance of certain factors: type of lesion, timing of tests, and contamination of amniotic fluid by fetal blood.

Anencephaly and spina bifida. The type of lesion which is more often associated with positive AFP assays than spina bifida is anencephaly.

The importance of timing is seen in the initial 37 cases of NTD of Brock and Sutcliffe.[118] They concluded that amniotic fluid measurements were valuable for early antenatal diagnosis because the concentration of AFP in spina bifida was four times greater than its normal level even at 13 weeks whereas, after 30 weeks, the difference between normal and abnormal levels of AFP was not as great. Levels should be followed on a standard or normal curves of values established for a given laboratory (Fig. 3).

Allan and associates[209] studied 190 samples of amniotic fluid in the second trimester by CIEP and confirmed the value of AFP detection in open neural tube defects sufficiently early to allow termination. Ultrasound studies were not as useful as AFP determinations at 16 weeks of gestation in two cases of spina bifida. Field and associates[210] reported a case of anencephaly which was missed by ultrasonic examination but detected by the radial immunodiffusion method for AFP at 14 to 16 weeks of gestation. Laurence and associates[201] reported a closed lesion (encephalocele) which had normal levels of AFP by radioimmunoassay at 16 weeks but an AFP concentration that was 10 times the upper limit of normal at 34 weeks. Detection of myeloceles at 6 weeks and 15 weeks by the Laurell rocket technique was reported by Nevin and associates.[211] The magnitude of elevation of AFP in NTD ranges from five to 10 or more times the upper limits of normal, depending on the type and combination of lesions.

Not only does anencephaly have a higher positivity rate of AFP results than spina bifida, but it is usually associated with higher levels. Although different AFP assays were used in different geographic locations, one should note that conclusions about NTD's are similar in reports by Milunsky and associates,[212] Randle and Cumberbatch,[213] Lorber and associates,[214] and Seller and associates.[92] All of these dealt with anencephaly either alone or in conjunction with other neural tube defects.

Hydrocephaly. Seppälä and Unnerus[215] reported the first case of elevated amniotic AFP in hydrocephaly in

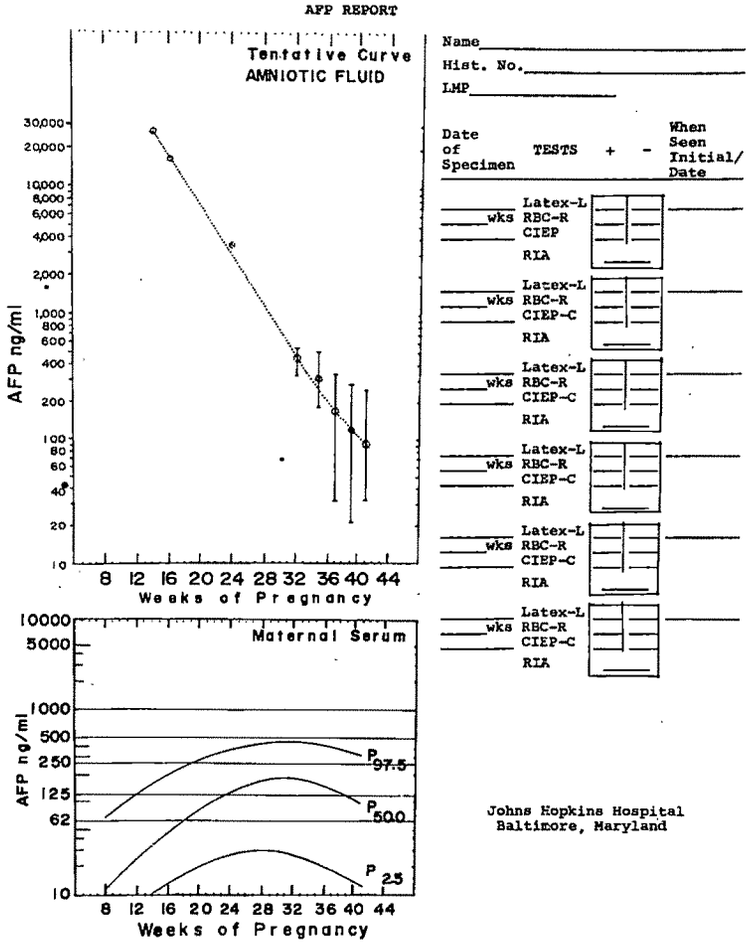


Fig. 3. Patient report sheet used at The Johns Hopkins Hospital.

May, 1974. They reminded their readers that elevated amniotic fluid AFP could be found in congenital fetal anomalies other than neural tube defects, such as congenital nephrosis and esophageal atresia.

Fetal death as a cause of AFP in anencephaly. Intrauterine fetal death as a cause of elevated amniotic fluid AFP levels in anencephaly rather than anencephaly per se has been considered in some cases.[212, 215]

Contamination. Contamination of amniotic fluid specimens by fetal blood giving false-positive results is very likely when one remembers that concentrations of AFP are expressed as milligrams per milliliter in fetal blood, as micrograms per milliliter in amniotic fluid, and nanograms per milliliter in maternal serum. Milford, Ward and Stewart[216] calculated that the volume of fetal blood which would elevate the amniotic fluid AFP above the norm is 1 ml. of fetal blood at 14 weeks of gestation, 2.5 ml. at 15 weeks and 5 ml. at 17 weeks. The actual volume of fetal blood necessary to elevate amniotic fluid AFP may be smaller than these figures due to incomplete mixing of fetal blood in amniotic fluid.

Several clinically important steps are suggested by these findings. Specimens should be tested for fetal red blood cells by the Kleihauer technique.[216] If the sample is contaminated by blood, AFP assays should not be done. Total protein as well as AFP should be measured in amniotic fluid to distinguish AFP leakage from an open lesion versus a fetal-maternal transfusion or intrauterine death.

The use of maternal serum in screening for neural tube defects by AFP levels progressed from a "guarded assessment" in initial reports to a "more promising" assessment based on additional reports in 1974 to a "high hopes" assessment by the time of a London workshop in May, 1974. Elevated levels of AFP in maternal serum were reported in cases of neural tube defects as early as 13, 16, and 21 weeks of gestation with the impression that the levels of AFP in maternal serum were not as clear-cut as those in amniotic

fluid.[199, 217] This impression may have been due to the initial difficulty in measuring nanogram quantities of AFP in maternal serum by radioimmunoassay. Because fetal serum levels were normal in seven anencephalic fetuses, no increased synthesis of AFP is believed to occur in fetuses with severe neural tube defects,[199] suggesting that the elevation of AFP in amniotic fluid and maternal serum is due to a leakage of AFP through exposed neural surfaces rather than an increased fetal synthesis. In comparing open versus closed lesions and AFP levels in amniotic fluid versus maternal serum, Harris and associates[200] pointed out that in determinations using amniotic fluid, no false positive results have been encountered so far. With maternal serum, AFP assays may miss very early neural tube defects, whether open or closed, but this may be at a clinically acceptable level of 10 per cent efficacy, as pointed out by Brock and associates.[218] They state that even at a level of efficacy of 10 per cent, screening a pregnant population by AFP assays in maternal serum would be justified in the United Kingdom because 200 fewer children with spina bifida would be born. A later controlled study,[219] which matched cases and controls on the basis of maternal age, parity, and length of gestation, concluded that the screening of maternal serum for AFP levels was useful in detecting neural tube defects, especially for anencephaly and spina bifida and especially for early pregnancy (below the twentieth week) when the difference between cases and controls was larger. An even more encouraging assessment on the value of maternal serum emerged from the London workshop.[220] Elevated levels of AFP were detected between the twentieth and fortieth weeks of gestation (the clinically useful interval) in 80 per cent of anencephalic and almost 60 per cent of fetuses with spina bifida. Two cases with closed lesions had levels at the upper limit of normal. The importance of maternal serum in contrast to amniotic fluid was explained by the previously mentioned practice in England whereby the risk of amniocentesis versus the risk of a neural tube defect justified amniocentesis only in those patients with a previous history of the disease. Although determinations of AFP in amniotic fluid could provide prenatal diagnosis for 90 per cent of spina bifida and 100 per cent of anencephaly in time for selective abortion, the practice of limiting amniocentesis to only a select high-risk group would in fact reduce the cases of spina bifida by only 10 per cent. Thus, maternal serum assays might provide a higher detection rate. Additionally, since closed lesions represent only 4 per cent of cases with neural tube defect which survive, the lack of AFP elevation in this small group may not affect the screening efficacy in maternal serum, since some of these will have less serious lesions.

Importance of using other tests and preventing abortions. The importance of using other tests, such as scanning sonograms and x-rays, to follow-up elevated AFP levels has been stressed.[199, 200] Termination of pregnancy in cases of neural tube defect substantiated by these other tests can reduce the risk of a living child with a serious disabling malformation from one in 10 to less than one in 100.[200]

AFP assays with amniotic fluid and maternal serum have been successfully used to screen for neural tube defects as early as 6 and 12 weeks of gestation, and clearly before the twentieth week, far in advance of the time when the lesions would be detectable by ultrasound scanning and x-ray examinations. Used in a prenatal diagnostic screening center, the use of AFP determinations in these fluids may prevent more abortions than they selectively indicate.

Fetal macrophages and AFP. The presence of fetal macrophages with elevated levels of AFP in amniotic fluid may increase the efficacy of amniocentesis in screening for neural tube defects, according to Sutherland[221] and Nelson[222] and their colleagues.

Treatment. Treatment of neural tube defects may be influenced by earlier identification of cases with AFP assays. The debate of whether to treat all affected infants surgically centers on a controversy of increased survival versus a poor quality of life in those who survive.[202, 223] Laurence[202] showed that of 100 liveborn cases, survival increased from 17 to 50 cases with operation, but that the number of cases dependent on wheelchairs and the number handicapped also increased. Increased effort toward antenatal detection of spina bifida was emphasized. In contrast to this suppression of poor quality of life, Crosby[224] in Baltimore has been advocating aggressive surgical treatment for a better quality of life in cases with spina bifida. He cited that many of these patients have above-average intelligence and when admitted to public schools have done exceedingly well. Here, again, early antenatal detection would be important, mainly to alert and emotionally prepare both parents and the surgical team.

Need for prospective studies. In spite of the large body of knowledge of AFP which has appeared since 1970, information is still needed about the applicability of AFP testing to routine prenatal care and about the types of birth defects other than NTD which are associated with abnormal AFP levels.

A prospective study is being conducted in the prenatal clinics of the Johns Hopkins Hospital to evaluate the application of AFP assays to routine prenatal care. A study of this sort had been suggested

by Seppälä, who had conducted prospective studies restricted to high-risk pregnancies and cases of threatened abortion.

Seppälä's study. In Seppälä's prospective study of 840 high risk pregnancies, 23 (2.74 per cent) AFP results were above the normal limit.[47] Of these, 12 were associated with fetal death, three with threatened abortion (all of which aborted), one abruptio placentae with intrauterine death at 28 weeks, one twin pregnancy in which one fetus was dead at 17 weeks, and four pregnancies complicated by Rh immunization, toxemia, hepatosis, and diabetes. In another prospective study of 112 women with threatened abortion, Seppälä[47] obtained six elevated AFP results (5.4 per cent), all of which were associated with eventual fetal death or abortion. In a prospective nonconcurrent study at Johns Hopkins Hospital,[94] seven abnormal AFP results were obtained in 298 maternal sera randomly collected from the prenatal clinic. Fetal death subsequently occurred in six cases and the seventh case was associated with severe toxemia and a depressed newborn infant. Important clinical features of this study which agree with Seppälä's findings are the highest level of maternal AFP found in fetal death (8,000 ng. per milliliter) compared to 9,000 ng. per milliliter in Seppälä's series) and a lack of antibodies against AFP in maternal serum.

Current experience. In the current prospective study at Johns Hopkins Hospital, AFP assays are being performed on every patient passing through the prenatal clinics until a sample size of 2,000 cases is reached. One thousand patients constitute the test group in which the AFP results are reported to the obstetric staff; the other thousand patients constitute a control group in which the AFP results are withheld from the obstetric staff. Patients in the test group will be matched with patients in the control group on the basis of age, race, parity, and socioeconomic status. Further matching will be done on the complications of pregnancy such as toxemia, diabetes, and Rh immunization. One purpose of the study is to see whether changes in obstetric management result from the presence of AFP results in comparison to their absence. Another purpose is to see whether differences between the two groups will occur in pregnancy outcome, such as rates of prematurity, induction, and cesarean section. Differences in fetal morbidity and mortality rates will be sought.

Another study being conducted at the same institution involves a different population of patients attending the prenatal diagnostic center, which provides genetic counseling. The purpose of this study is to see whether the addition of AFP assays to the diagnostic tests available aids in the diagnosis and prevention of birth defects. Another purpose of this study is to collect more data about malformations in early pregnancy, which is either insufficient or totally lacking, as stated by Seppälä.[47]

In both studies, three "simplified" assays and a double-antibody radioimmunoassay are being performed on each sample of maternal serum or amniotic fluid. The purpose is to compare the ease of performance, the reliability, and the clinical value of each test. To date, the radioimmunoassay has provided a direct quantification of AFP levels but has the disadvantage of a delay of 2 weeks, which may hinder or delay clinical action as a result of an AFP report. Additionally, a gamma counter and refrigerated centrifuge are necessary to set up the RIA, making the cost prohibitive for a small laboratory. The three simplified assays are CIEP (counterimmunoelectrophoresis), LS (a direct latex slide test), and an RBC (a direct red cell test). Initially, the CIEP was expected to be the more cumbersome test, since the agarose slides had to be prepared daily, in contrast to the LS and RBC test reagents, which can be made up ahead of time and stored. After almost a year, it appears that the CIEP is the easiest to perform and the most reliable of the simplified assays. It gives no dubious end points and the amount of AFP can be quantified both by comparing the precipitin line with standards and by a distance of displacement from the antibody well. The sensitivity of the assay has improved from 250 to 125 ng. per milliliter and occasionally even 62.5 ng. per milliliter when compared to purified standard. By contrast, the latex slide test, although easy to perform, gives dubious end points, some of which can be clarified by an absorbant. The sensitivity of this assay is 250 ng. per milliliter. The ease of performance of this test, however, remains a highly desirable feature. The red cell test gives less dubious end points than the latex test but has the disadvantage of a 1 hour interval for the rings to settle out. The sensitivity is 16 to 32 ng. per milliliter, requiring dilution of the serum to obtain satisfactory results. The combination of two or more of these assays may provide the optimal testing facility for AFP screening; but the ultimate answer awaits the completion of this study and a statistical evaluation. To date, 2,222 maternal sera and 106 amniotic fluid samples have been obtained over 9 months. Of the maternal sera, 222 (9.9 per cent) have shown elevated levels; 16 (15.1 per cent) of the amniotic fluid samples have shown elevated levels. The results are plotted on tentative "normal" curves modified from data published by Seppälä.[129, 197] These

curves are shown in Fig. 3. In a few cases of the test group, levels assessed as abnormally high according to his criteria correlated well with clinical findings.

In one case, a patient who developed hydramnios was associated with rising AFP values in both maternal serum and amniotic fluid. The patient, a 28-year-old, white woman, para 2-0-0-1, had a second pregnancy marked with hydramnios with delivery of a male infant with spina bifida, meningomyelocele, and hydrocephalus. Due to her previous history, amniotic fluid and maternal serum were obtained at 20 weeks. The AFP levels were 9,234 ng. per milliliter in amniotic fluid and 45 ng. per milliliter in maternal serum, both within normal limits. Hydramnios developed later and at 36 weeks amniotic fluid and maternal serum were again obtained. The values were 1,249 and 224 ng. per milliliter respectively. The AFP amniotic fluid value is elevated and the serum value in the upper normal range. At birth, a 1,930 gram female infant was delivered with micropthalmia, microstomia, micrognatha, large cleft of soft palate, and arthrogryposis. No anencephaly or spina bifida was present.

In another case, normal AFP values in *maternal serum* were obtained throughout pregnancy. At term, however, a male infant with numerous anomalies suggestive of cebocephaly was delivered. The value of an amniotic fluid AFP determination would have been immeasurable in determining its predictive value for this condition. The patient, a 23-year-old black woman, para 3-0-0-3, had numerous complications in this pregnancy, including excessive weight gain, high blood pressure, gestational diabetes, and dates-examination discrepancy. The family history included three sisters with thyroid disease and one sister with spina bifida. The AFP values in maternal serum were 79 ng. per milliliter at 19 weeks, 245 ng. per milliliter at 30 weeks, 305 ng. per milliliter at 32 weeks, and 239 ng. per milliliter at 33 weeks. A 2,610 gram male infant was delivered with numerous anomalies associated with cebocephaly.

In the near future, results of large-scale prospective field trials evaluating the application of AFP testing to routine obstetric-gynecologic care and cancer screening should appear in the literature. These reports should delineate further *when, where,* and *how* AFP assays are to be used in the future.

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ITEMS

Advanced course in colposcopy with video tape tutorial, July 22-24, 1976

An advanced course in colposcopy will be held during Montreal's Summer Olympics on July 22-24, 1976, at the Jewish General Hospital, Montreal, Quebec, Canada. Invited speakers include Drs. Duane Townsend, Arthur Herbst, Adolph Stafl, and Ralph Richart.

The registration fee is $250 and includes advanced colposcopy, a session with a live patient, video tape tutorial, and lunches. Registration must be made by December 15, 1975, and should be accompanied by a check payable to: Alex Ferenczy, M.D., Registrar, Jewish General Hospital, Department of Pathology, 3755 Cote St. Catherine Rd., Montreal, H3T 1E2, Quebec, Canada.

First International Congress on the Menopause

The First International Congress on the Menopause will be held June 8 to 11, 1976, at La Grande Motte, a seaside resort south of Montpellier, France, under the auspices of the American Geriatric Society and the Medical Faculty of the University of Montpellier. This meeting will consist of a limited number of workshops on selected important topics and sessions with free communications. The Organizing Committee has invited a number of investigators to participate in the workshops, but it will also be possible to accommodate a limited number of observers (as well as participants in the free communications sessions). The total amount of participants will not exceed 200 people.

Requests for registration or for additional information should be sent as soon as possible to the Program Co-ordinator: Dr. P. A. van Keep, c/o International Health Foundation, 1 Place du Port, 1204 Geneva, Switzerland. Upon confirmation of the registration, a fee of $50.00 will be due. Those wishing to present a paper in the free communications sessions should submit an abstract for consideration as early as possible to the same address.

Conference on psychosomatic obstetrics and gynecology

"Practical Applications of Psychosomatic Obstetrics and Gynecologic Concepts to Patient Care" will be the theme of the conference sponsored by the United States and Canadian Sections of the International Society of Psychosomatic Obstetrics and Gynecology to be held Friday afternoon and evening, April 9, and Saturday, April 10, 1976, in Chicago.

Topics discussed will include: (1) Psychological, Social, and Spiritual Care of Patients with Gynecologic Cancer; (2) Techniques of Hysterectomy Counseling; (3) Methods of Improving the Experience of the Mother and Baby in the Postpartum Period; (4) The Role of the Family and Consumer Groups in the Delivery of Psychosomatic Obstetric-Gynecologic Care.

The meeting will take place at The University of Chicago Center for Continuing Education which provides retreat-type surroundings with conference rooms, meal facilities, and guest rooms under one roof. For registration information and complete program, write to: Toby Hofslund, 1307 E. 60th St., Chicago, Illinois 60637. Telephone: (312)753-3189.

Sixteenth Annual Meeting of the Teratology Society

The Teratology Society will hold its Sixteenth Annual Meeting June 20-23, 1976, at Highlands Inn, Carmel, California. For further information, write: Lucille S. Hurley, Ph.D., Department of Nutrition, University of California, Davis, California 95616.

VIII World Congress of Gynecology and Obstetrics

The VIII World Congress of Gynecology and Obstetrics will be held in Mexico City, Mexico, October 17-22, 1976.

Social program (for participants and accompanying persons).

Sunday, October 17, 8:00 P.M., Welcome Reception at the National Museum of Anthropology, a contemporary building that houses the art treasures of pre-Columbian Mexico.

Monday and Tuesday, October 18 and 19, 8:00 P.M., performance of the Ballet Folklórico de México at the Palace of Fine Arts. Same program on both days. Admission limited to capacity of theater.

Wednesday, October 20, 8:00 P.M., Mexican "fiesta" in the patio of the "Las Vizcainas" building, an interesting eighteenth century baroque construction.

Thursday, October 21, 8:00 P.M., concert at the Metropolitan Cathedral, a building that incorporates the five distinctive architectural styles of the colonial period.

Friday, October 22, 9:00 P.M., Official Banquet. It is

suggested that tickets be ordered in advance (see form 1) at a price of $25.00 per person. Limited admission.

Ladies' program. In addition there will be the following activities especially organized for accompanying persons:

Monday and Tuesday, October 18 and 19, morning, sightseeing tour, including the most interesting and picturesque spots in Mexico City. Same tour on both days.

Thursday, October 21, morning and afternoon, visit to the Museo Nacional del Virreinato at the former San Martin de Tepotzotlán monastery, a gem of eighteenth century colonial architecture located at a distance of 44 km. from the city. Lunch included.

For further information, write: General Secretariat, Avenida Baja California 317, México 11, D. F., Mexico.

Twelfth Annual E. C. Hamblen Symposium in Reproductive Endocrinology and Perinatal Medicine

The Department of Obstetrics and Gynecology, Duke University Medical Center, Durham, North Carolina, announces the "Twelfth Annual E. C. Hamblen Symposium in Reproductive Endocrinology and Perinatal Medicine," to be held March 19 and 20, 1976. Major topics are: "Metabolic Diseases in Pregnancy" and "Teratogenicity." Invited speakers include Dr. Edward Bishop, Chapel Hill, North Carolina, Dr. Rubin Bressler, Tucson, Arizona, and Dr. William Spellacy, Gainesville, Florida.

For further information, contact: Dr. Charles B. Hammond, P. O. Box 3143, Duke University Medical Center, Durham, North Carolina 27710.

1976 Conference on Clinical Delineation of Birth Defects

The University of British Columbia, Faculty of Medicine, and the National Foundation–March of Dimes, will hold its 1976 Conference on Clinical Delineation of Birth Defects, June 23-25, 1976. For information, write: Dr. R. Brian Lowry, Clinical Genetics Unit, 855 W. 10th Ave., Vancouver, British Columbia V5Z 1L7, Canada.

Current Concepts of Infections in Obstetrics and Gynecology

The Department of Obstetrics and Gynecology of William Beaumont Hospital announces its Second Annual Symposium, "Current Concepts of Infections in Obstetrics and Gynecology," to be held March 26 and 27, 1976.

Nationally recognized authorities will discuss: (1) Infectious Factors in Perinatal Loss, (2) Infectious Disease Management in the Pregnant Woman, and (3) Management of Pelvic Infections in the Gynecologic Patient. The distinguished guest faculty will consist of Drs. William Ledger, Abe Mickal, Richard Sweet, and Paul Wiesner. Registration fees: $100.00 for practicing physicians and $50.00 for physicians in training. Attendance will be limited to 100.

For further information, contact: Department of Obstetrics and Gynecology, William Beaumont Hospital, 3601 W. 13 Mile Rd., Royal Oak, Michigan 48072.

Gynecologic Surgery and Pathophysiology

The Department of Obstetrics and Gynecology of The George Washington University Medical Center and the Kane-King Obstetrical Society announce a postgraduate course entitled "Gynecologic Surgery and Pathophysiology," to be held April 1-3, 1976. Guest faculty will include Dr. Robert W. Kistner, Harvard Medical School, Dr. John McL. Morris, Yale University School of Medicine, and Dr. Richard Symmonds, Mayo School of Medicine.

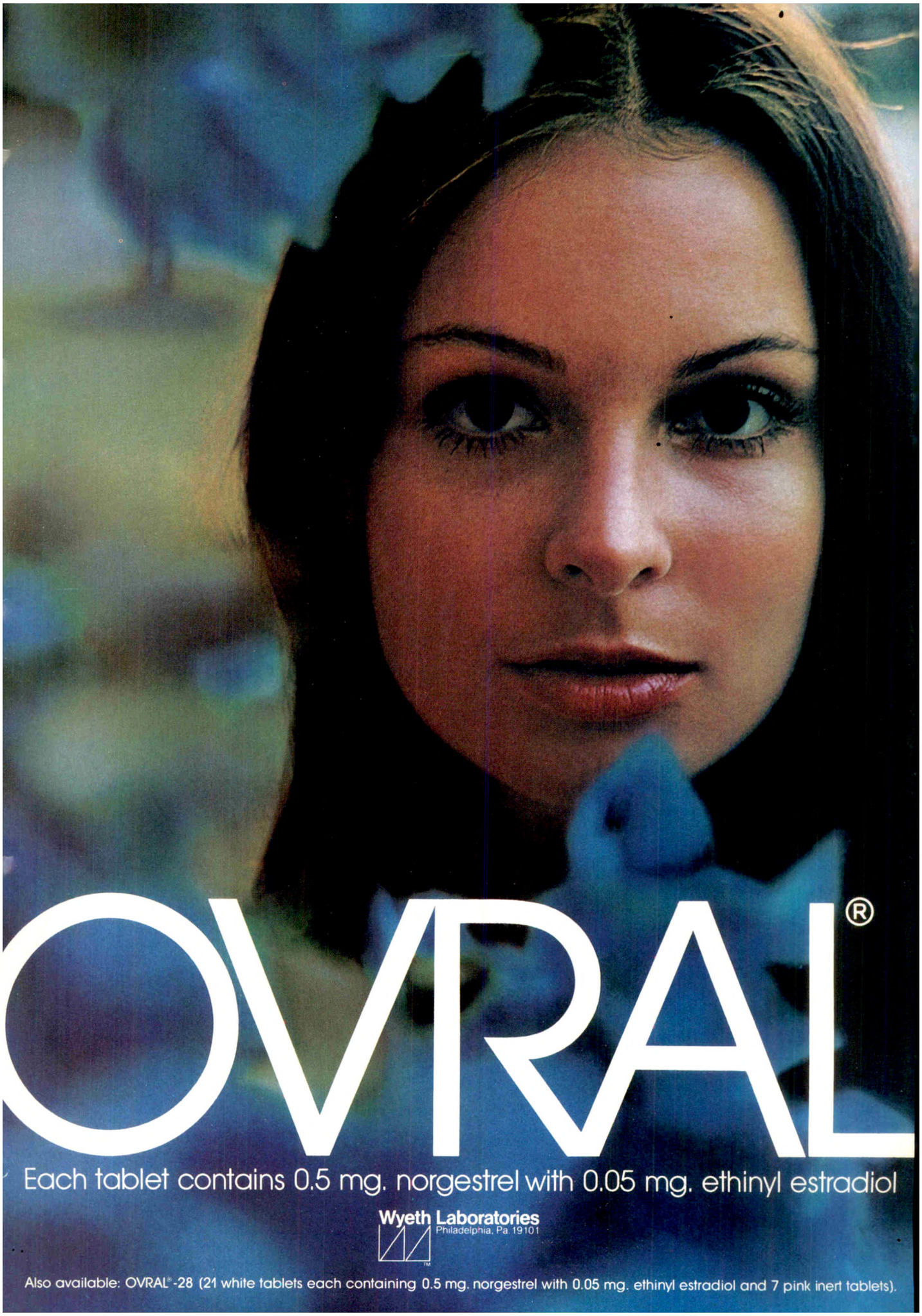
This program has been approved for Physicians Recognition Award Category 1 (14 hours). For additional information, please contact: Dr. Allan B. Weingold, Department of Obstetrics and Gynecology, The George Washington University Medical Center, 2150 Pennsylvania Ave., N.W., Washington, D. C. 20037.

Gynecologic Oncology: Postgraduate seminar and colposcopy course

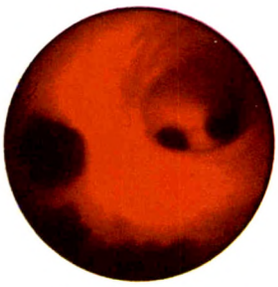
The Division of Gynecologic Oncology, Crawford W. Long Memorial Hospital of Emory University, announces a postgraduate course in "Gynecologic Oncology" which will be held April 21-24, 1976, and will include the following: (1) a two-day seminar on "Carcinoma of the Female Genital Tract," including a review of current therapy, immunology, and infection; (2) a two-day course of instruction in "Basic and Advanced Colposcopy," including lectures, patient demonstration, and video tape instruction. Guest faculty will include Robert Scully, M.D., Ralph Richart, M.D., Adolf Stafl, M.D., Ph.D., Duane Townsend, M.D., Taylor Wharton, M.D., Philip DiSaia, M.D., James Reagan, M.D., and William Ledger, M.D. For brochure and application, write: Ernest W. Franklin III, M.D., Gynecologic Oncology, 25 Prescott St., N.E., Atlanta, Georgia 30308.

XXIVth Annual Colloquium on Protides of the Biological Fluids

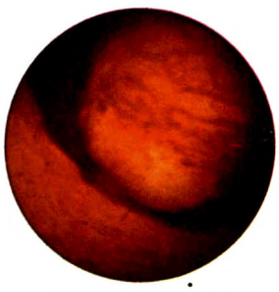
The XXIVth Annual Colloquium on Protides of the Biological Fluids will be held in Brugge, Belgium, April 27–May 1, 1976. The following topics will be covered during the meeting: (1) pregnancy-associated proteins, (2) carcino-fetal and carcino-placental proteins, and (3) detection and quantitation of proteins by molecular amplification. All correspondence should be sent to the Secretariate: XXIV Colloquium "Protides of the Biological Fluids," Simon Stevin Instituut—Jerusalemstraat 34, B-8000 Brugge, Belgium. Telephone: 050/33.45.34. Telex: 81 379 SSI B.


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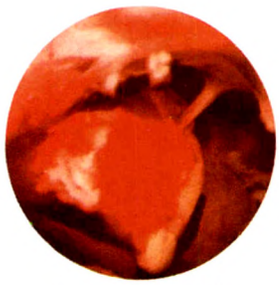
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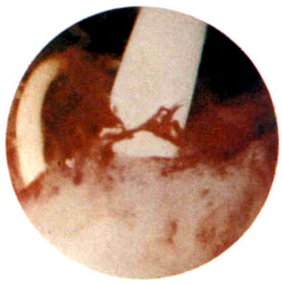
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Hyskon has a tendency to crystallize when subjected to temperature variations or when stored for long periods. If flakes of dextran are present, heat at 100°-110° C until complete dissolution is achieved.

INDICATIONS: Hyskon Hysteroscopy Fluid is indicated for use with the hysteroscope as an aid in distending the uterine cavity and in irrigating and visualizing its surfaces.

CONTRAINDICATIONS: Hyskon Hysteroscopy Fluid should not be instilled in patients known to be hypersensitive to dextran. All other contraindications are those relative to the hysteroscopic procedure itself, such as pregnancy, endometrial carcinoma, etc.

WARNINGS: It is not known whether systemic absorption of dextran 70 occurs from the uterine and peritoneal cavities. Because of the possibility that absorption can occur, the physician should familiarize himself with the systemic effects of this drug. The most alarming are severe, sometimes fatal anaphylactoid reactions, which are quite rare. Other noteworthy effects include plasma volume expansion and transient prolongation of bleeding time.

ADVERSE REACTION: Undesirable effects directly attributable to the use of Hyskon Hysteroscopy Fluid have not been reported. However, the physician should be mindful of the fact that adverse reactions are known to occur with the intravenous administration of dextran 70 (MACRODEX 6% W/V). Such reactions have included: allergic phenomena (anaphylactoid reactions, urticaria, nasal congestion, wheezing, tightness of chest and mild hypotension), nausea, vomiting, fever and joint pains.

DOSAGE AND ADMINISTRATION: The amount of Hyskon Hysteroscopy Fluid required per patient depends on a number of factors, such as the type and length of the diagnostic procedure, whether or not manipulation or surgery is performed, etc. Most often, however, the amount of Hyskon instilled into the uterus will be between 50 and 100 milliliters.

Hyskon should be introduced into the uterine cavity through the cannula of a hysteroscope under low pressure (approximately 100 mm Hg) until the uterus is sufficiently distended to permit adequate visualization. During the hysteroscopic examination, Hyskon should be infused at a rate that keeps the cavity suitably distended. To avoid injection of the fluid into the tissues of the uterus and parametria and to avoid having unnecessary amounts of the fluid pass into the peritoneal cavity and backwards along the side of the hysteroscope, infusion pressures greater than 150 mm Hg should not be used.

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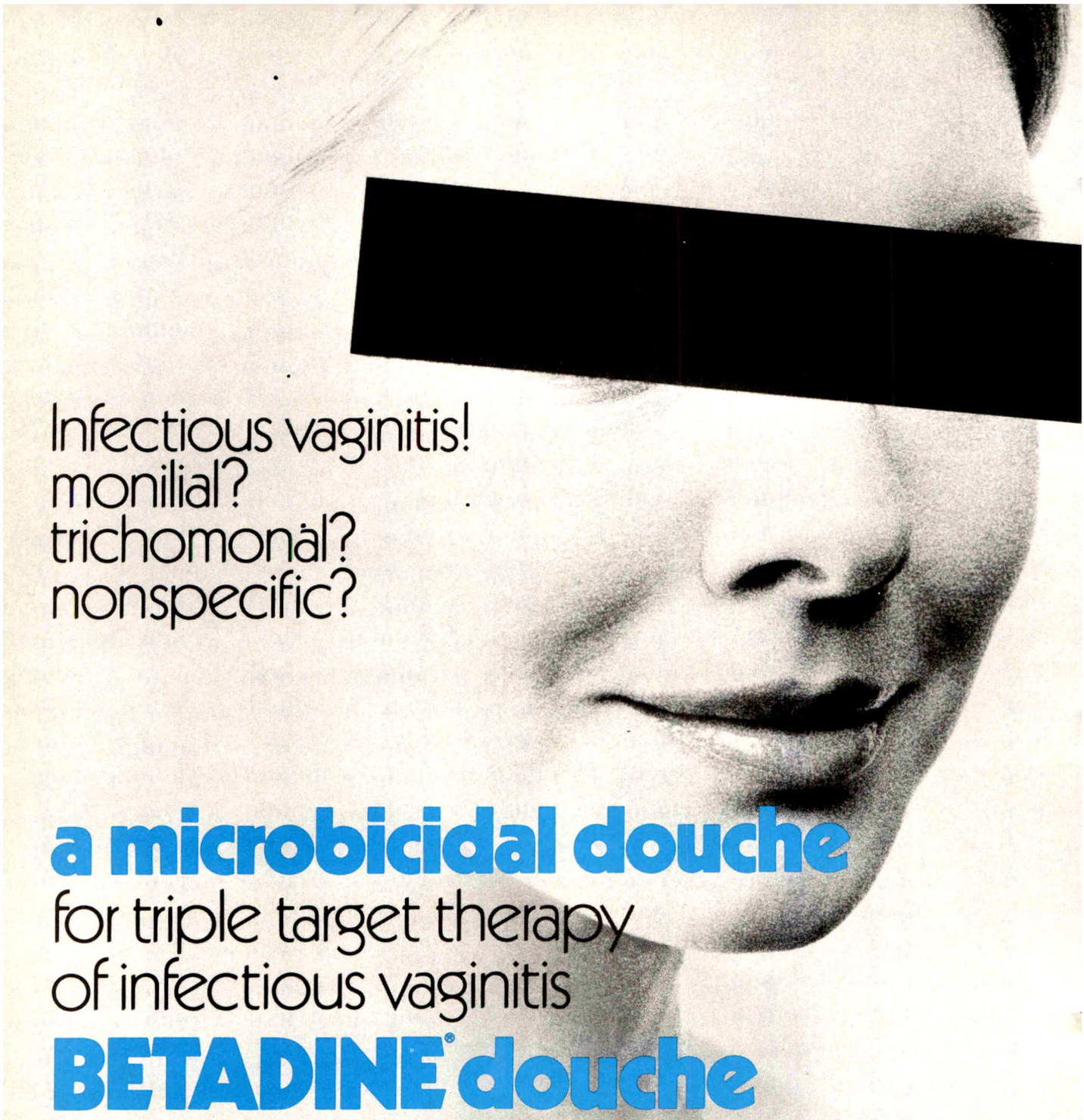
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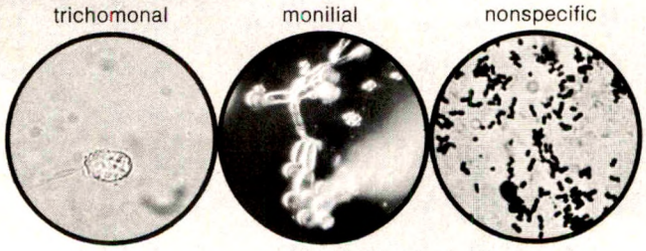
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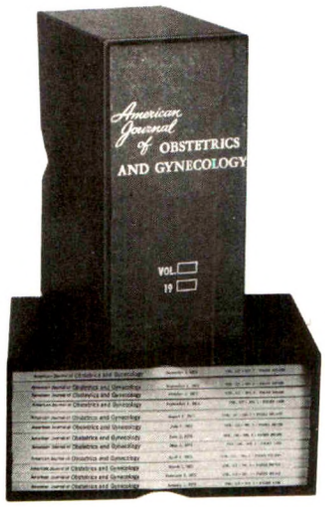
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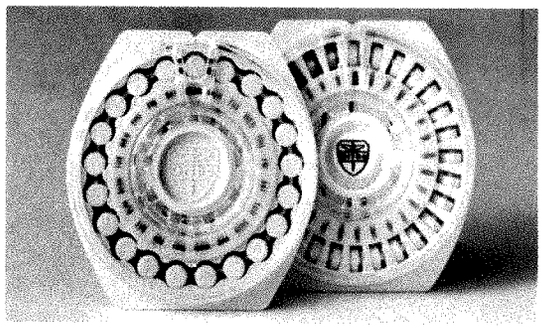
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Description: Each ORTHO-NOVUM 1/50 ◻21 Tablet contains 1 mg of the progestational substance, norethindrone, together with 0.05 mg of the estrogenic compound, mestranol.

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Actions: ORTHO-NOVUM 1/50 ◻21 and ORTHO-NOVUM 1/50 ◻28 act through the mechanism of gonadotrophin suppression, i.e., through the estrogenic and progestational actions of the ingredients. Alterations in cervical mucus and in the endometrium may also contribute to the efficacy of ORTHO-NOVUM 1/50 ◻21 and ORTHO-NOVUM 1/50 ◻28.

Special note: Oral contraceptives have been marketed in the United States since 1960. Reported pregnancy rates vary from product to product. The effectiveness of the sequential products appears to be somewhat lower than that of the combination products. Both types provide almost completely effective contraception.

The physician should consider a thorough discussion with each patient about the prescribing information, risks and benefits associated with oral contraceptives, taking into account various factors such as age and other physical conditions.

An increased risk of thromboembolic disease associated with the use of hormonal contraceptives has now been shown in studies conducted in both Great Britain and the United States. Other risks, such as those of elevated blood pressure and reduced tolerance to carbohydrates, have been reported and appropriate tests should be conducted to monitor these during oral contraceptive therapy. Liver disease has also been reported, and the physician should be alert to its earliest manifestations.

Long-term administration of both natural and synthetic estrogens in subprimate animal species in multiples of the human dose increases the frequency for some animal carcinomas. These data cannot be transposed directly to man. The possible carcinogenicity due to the estrogens can neither be affirmed nor refuted at this time. Close clinical surveillance of all women taking oral contraceptives must be continued.

Indication: Contraception.

Contraindications: 1. Thrombophlebitis, thromboembolic disorders, cerebral vascular disease, myocardial infarction, or a past history of these conditions. 2. Markedly impaired liver function. 3. Known or suspected carcinoma of the breast. 4. Known or suspected estrogen-dependent neoplasia. 5. Undiagnosed abnormal genital bleeding. 6. Known or suspected pregnancy.

Warnings: 1. The physician should be alert to the earliest manifestations of thrombotic and thromboembolic disorders, thrombophlebitis, cerebrovascular disorders including hemorrhage, pulmonary embolism and retinal thrombosis. Should any of these occur or be suspected, the drug should be discontinued immediately.

Retrospective studies of morbidity and mortality in Great Britain and studies of morbidity in the United States have shown a statistically significant association between thrombophlebitis, pulmonary embolism and cerebral vascular disease, occlusive or hemorrhagic, and the use of oral contraceptives. There have been three principal studies in Great Britain[1-3] leading to these conclusions and three in this country.[4-7] The estimate of the relative risk of thromboembolism in the study by Vessey and Doll[3] was about seventold, while the United States studies found relative risks of 4.4 to 11, meaning that the users are several times as likely to undergo thromboembolic disease without evident cause as nonusers.

In May, 1974, the Royal College of General Practitioners in England[8] issued an interim report of its continuing large-scale prospective study comparing a user group to a nonuser group. This study in its interim analysis states, "A statistically significant higher rate of reporting of cerebrovascular accidents in Takers is evident, but the numbers are too small to justify an estimation of the degree of risk." The study also reported a higher incidence of superficial and deep vein thrombosis in users as compared to nonusers. The risk of superficial and deep vein thrombosis was reported to be lower in women using 50 mcg estrogen preparations.

The Sartwell study[4] indicated that the risk did not persist after discontinuation of administration. Both the Sartwell and the Royal College studies indicated that the degree of risk was not associated with duration of treatment.

In a collaborative American study[5,6] of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the relative risk of thrombotic stroke was 4.1 to 9.5 times greater in users than in nonusers. A comparable estimate for hemorrhagic stroke was 2.0.

None of the American studies was designed to evaluate a difference between products. However, the Sartwell study[4] suggested that there might be an increased risk of thromboembolic disease in users of sequential products.

Other retrospective studies[9,10] have reported an increased risk of post-surgery thromboembolic complications in oral contraceptive users. It has been recommended that therapy be discontinued at least one month prior to elective surgery.

In May of 1975 two separate retrospective case control studies relating to fatal and nonfatal myocardial infarction in users and nonusers of oral contraceptives were reported.[11,12] The studies estimate that the increased risk of hospital admission for myocardial infarction associated with the use of oral contraceptives as compared to nonusers is 2.7 times in the 30-39 age group and 5.7 times in the 40-44 age group; and the risk of death associated with the use of oral contraceptives as compared to nonusers was increasec 2.8 times for users in the 30-39 age group and 4.7 times in the 40-44 age group. One of these studies reported that oral contraceptive users who had myocardial infarction were more likely to be heavy smokers and have certain additional risk factors, i.e., treated hypertension and diabetes, pre-eclamptic toxemia, obesity and type II hyperlipoproteinemia.

2. Discontinue oral contraceptive medication if there is: gradual or sudden partial or complete loss of vision; proptosis or diplopia; onset or aggravation of migraine or development of headache of a new pattern which is recurrent, persistent or severe; papilledema; or any evidence of retinal vascular lesions.

3. Fetal abnormalities have been reported to occur in the offspring of women who have taken progestogens and/or estrogens during pregnancy.[13,14] The safety of ORTHO-NOVUM 1/50 ◻21 or ORTHO-NOVUM 1/50 ◻28 in pregnancy has not been demonstrated. Pregnancy should be ruled out before initiating or continuing the contraceptive regimen. Pregnancy should always be considered if withdrawal bleeding does not occur.

4. A small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The long-range effect to the nursing infant cannot be determined at this time.

5. Hepatic lesions (adenomas, hepatomas, hamartomas, regenerating nodules, etc.), occasionally fatal, have been reported in women on oral contraceptives. Such lesions may present as an abdominal mass or with the signs and symptoms of an acute abdomen. These lesions should be considered if the patient has abdominal pain or evidence of intra-abdominal bleeding. This has been reported in short-term as well as long-term users of oral contraceptives.

Precautions: 1. A thorough history and physical examination should be performed before prescribing oral contraceptives and periodically during their administration and should include special reference to breasts and pelvic organs, including Papanicolaou smear since estrogens have been known to produce tumors, some of them malignant, in five species of subprimate animals. 2. Endocrine and possibly liver function tests may be affected by treatment with ORTHO-NOVUM 1/50 ◻21 or ORTHO-NOVUM 1/50 ◻28. Therefore, if such tests are abnormal in a patient taking ORTHO-NOVUM 1/50 ◻21 or ORTHO-NOVUM 1/50 ◻28, it is recommended that they be repeated after the drug has been withdrawn for two months. 3. Under the influence of estrogen-progestogen preparations, pre-existing uterine fibromyomata may increase in size. 4. Because these agents may cause some degree of fluid retention, conditions which might be influenced by this factor, such as epilepsy, migraine, asthma, cardiac or renal dysfunction, require careful observation. 5. In breakthrough bleeding, and in all cases of irregular bleeding per vaginam, nonfunctional causes should be borne in mind. In undiagnosed bleeding per vaginam, adequate diagnostic measures are indicated. 6. Patients with a history of psychic depression should be carefully observed and the drug discontinued if the depression recurs to a serious degree. 7. Any possible influence of prolonged ORTHO-NOVUM 1/50 ◻21 or ORTHO-NOVUM 1/50 ◻28 therapy on pituitary, ovarian, adrenal, hepatic or uterine function awaits further study. 8. A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. The mechanism of this decrease is obscure. For this reason, diabetic patients should be carefully observed while receiving ORTHO-NOVUM 1/50 ◻21 or ORTHO-NOVUM 1/50 ◻28 therapy. 9. The age of the patient constitutes no absolute limiting factor, although treatment with ORTHO-NOVUM 1/50 ◻21 or ORTHO-NOVUM 1/50 ◻28 may mask the onset of the climacteric. 10. The pathologist should be advised of ORTHO-NOVUM 1/50 ◻21 or ORTHO-NOVUM 1/50 ◻28 therapy when relevant specimens are submitted. 11. Susceptible women may experience an increase in blood pressure following administration of contraceptive steroids. 12. Cholestatic jaundice has been reported in users of oral contraceptives. If this occurs, ORTHO-NOVUM 1/50 ◻21 or ORTHO-NOVUM 1/50 ◻28 should be discontinued. This condition is more likely to occur in patients who have experienced cholestatic jaundice of pregnancy. Patients with a history of cholestatic jaundice of pregnancy should be carefully observed during ORTHO-NOVUM 1/50 ◻21 or ORTHO-NOVUM 1/50 ◻28 therapy.

Adverse reactions observed in patients receiving oral contraceptives: A statistically significant association has been demonstrated between use of oral contraceptives and the following serious adverse reactions: thrombophlebitis, pulmonary embolism, cerebral thrombosis and hemorrhage, myocardial infarction, gallbladder disease.

Although available evidence is suggestive of an association, such a relationship has been neither confirmed nor refuted for the following serious adverse reactions: neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis; hepatic lesions with or without intra-abdominal bleeding.

The following adverse reactions are known to occur in patients receiving oral contraceptives: nausea, vomiting, gastrointestinal symptoms (such as abdominal cramps and bloating), breakthrough bleeding, spotting, change in menstrual flow, amenorrhea during and after treatment, edema, chloasma or melasma, which may persist, cholestatic jaundice, migraine, rash (allergic), mental depression, change in weight (increase or decrease), breast changes (tenderness, enlargement and secretion), changes in cervical erosion and cervical secretions, suppression of lactation when given immediately post-partum, rise in blood pressure in susceptible individuals.

Although the following adverse reactions have been reported in users of oral contraceptives, an association has been neither confirmed nor refuted: anovulation post-treatment, which tends to occur more frequently in women with a history of menstrual disorders, premenstrual-like syndrome, changes in libido, changes in appetite, cystitis-like syndrome, headache, intolerance to contact lenses, nervousness, dizziness, fatigue, backache, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruptions, itching, vaginitis.

The following laboratory results may be altered by the use of oral contraceptives: hepatic function—increased sulfobromophthalein retention and other tests; coagulation tests—increase in prothrombin, Factors VII, VIII, IX and X; decrease in anti-thrombin III; increase in platelet aggregability; thyroid function—increase in PBI, and butanol-extractable protein-bound iodine and decrease in T^3 uptake values; metyrapone test; pregnanediol determination.

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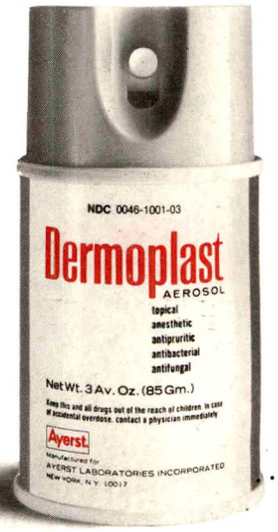
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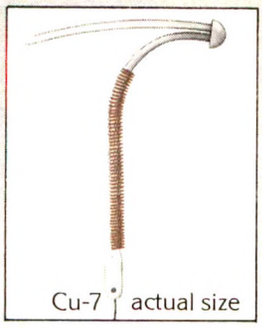
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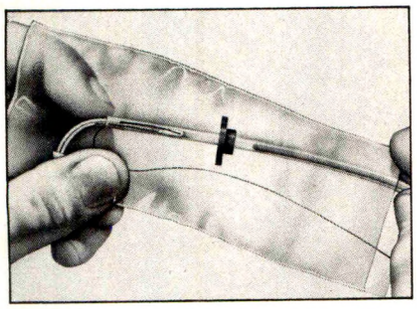
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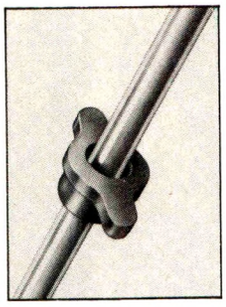


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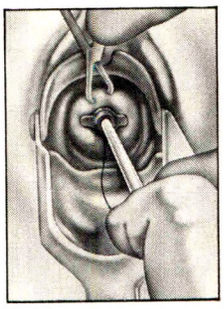
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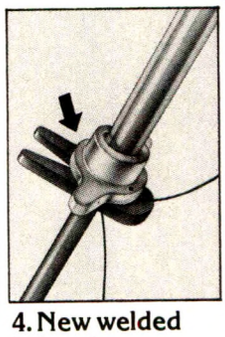
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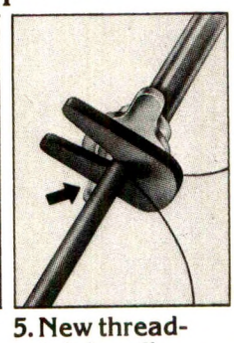
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Cu-7 (brand of intrauterine copper contraceptive)
Description: The plastic component of the Cu-7 is composed of pharmaceutical grade polypropylene homopolymer with barium sulfate added to render it radiopaque. Its shape approximates the number 7; it is substantially smaller than previously available intrauterine devices.

Coiled around the vertical limb is 89 mg. of pure, virgin electrolytic copper wire providing 200mm^2 of exposed copper surface area. A polypropylene retrieval thread is fastened to the free end of the vertical limb of the Cu-7.

The Cu-7 is supplied with a simple tubular polypropylene inserter. All components are sterile.

Action: Available data indicate that the contraceptive effectiveness of the Cu-7 is enhanced by a minute quantity of copper released continuously from the copper coil into the uterine cavity. The exact mechanism by which metallic copper enhances the contraceptive effect of an IUD has not been conclusively demonstrated. Various hypotheses have been advanced, the most common being that copper placed in the uterus interferes with enzymatic or other processes that regulate blastocyst implantation.

Animal studies suggest that copper may play an additional role by reduction of sperm transport within the uterine environment.

Indication: The Cu-7 is indicated for contraception.

Contraindications: The presence of any of the following, at the time of insertion, constitutes a contraindication: pregnancy, abnormalities of the uterine cavity, acute pelvic inflammatory disease or a history of repeated pelvic inflammatory disease, postpartum endometritis or infected abortion in the past three months, endometrial disease such as hyperplasia or carcinoma and a known or suspected allergy to copper.

Warnings: Recent reports suggest an increased incidence of septic abortion associated in some instances with septicemia, septic shock and death in patients becoming pregnant with an IUD in place. In some cases, initial symptoms have been insidious and not easily recognized. If pregnancy should occur with a Cu-7 in situ, the Cu-7 should be removed if the string is visible or, if removal proves to be or would be difficult, interruption of the pregnancy should be considered and offered as an option. If the patient elects to maintain the pregnancy and the Cu-7 remains in situ, she should be warned of the suggested incidence of increased risk of sepsis and followed with close vigilance.

The long-term effects on the offspring of the presence of copper in the uterus during pregnancy are unknown.

Perforations of the uterus have occurred. In this case the device should be removed immediately.

The use of microwave therapy in patients with metal prosthetics may cause heat injury to the surrounding tissue. Therefore, microwave therapy to the abdominal and sacral areas should not be used on patients wearing a Cu-7.

Additional amounts of copper available to the body from the Cu-7 may precipitate symptoms in women with undiagnosed Wilson's disease, the incidence of which is one in two hundred thousand.

Precautions: A pelvic examination should be done.

Sound the uterus prior to insertion and exercise care to avoid perforation. Do not use excessive force. The possibility of insertion in the presence of an existing undetermined pregnancy is reduced if insertion is performed shortly following a menstrual period. The Cu-7 should not be inserted post partum or post abortion until involution of the uterus has been completed.

Occasional perforations of the uterus (.03%) have been reported, usually during insertion into patients less than two months post abortion or post partum. If penetration into abdominal cavity occurs, laparotomy should be performed and the device recovered. Local inflammatory reaction with abscess formation is a possibility if a device is left in the abdomen.

If any patient with a Cu-7 suddenly develops overt clinical hepatitis or abnormal liver function tests, appropriate diagnostic procedures should be initiated.

The Cu-7 should be removed if pelvic infection which is unresponsive to treatment occurs.

The device should be replaced every two years. There is no evidence of decreasing effectiveness with time before two years, but the contraceptive effectiveness of the Cu-7 after two years has yet to be established.

The patient should be told that some light bleeding or cramps may occur during the first few days after insertion, but if these symptoms continue or are severe she should report to her physician. She should be instructed on how to inspect periodically to make certain that the thread still protrudes from the cervix and cautioned that there is no protection if the device has been expelled. She should also be cautioned not to pull on the thread and displace the Cu-7 and told to return in two years for its replacement.

A report has appeared in the literature suggesting that a copper-induced urticarial allergic skin reaction developed in a woman wearing a copper IUD. If symptoms of such an allergic response occur the patient should be instructed to tell the consulting physician that a copper-bearing device is being worn.

Adverse Reactions: Perforation of the uterus has occurred.

The incidence of spontaneous abortion, when conception occurs with intrauterine devices in situ, appears to be increased over that in unprotected women.

Insertion cramping, usually of no more than a few seconds' duration, may occur; however, some women may experience residual cramping for several hours or even days. Transient spotting or bleeding or prolongation of menstrual flow may occur in the first few cycles. Uncommonly, pelvic infection has been reported. Complete or partial expulsion may sometimes occur, particularly in those patients with uteri measuring less than 6.5 cm. by sounding. The following complaints have also been reported, although their relation to the Cu-7 has not been established: amenorrhea or delayed menses, backaches, cervical erosion, cystic masses in pelvis, vaginitis, leg pain or soreness, weight loss or gain, nervousness, dyspareunia, and cystitis.

Dosage and Administration: A single Cu-7 is to be inserted into the uterine cavity (see Precautions section). The small diameter of the Cu-7 enables easy insertion into the nulliparous as well as the multiparous uterus. Present information indicates that the efficacy is retained for at least 24 months. Until accurate data indicating a longer effective life become available, the Cu-7 should be removed and a new one inserted on or before 24 months from the date of insertion. If partial expulsion occurs, removal is indicated, and a new Cu-7 may be inserted. Removal of the Cu-7 may also be indicated in the event of heavy or persistent bleeding.

How Supplied: Available in cartons containing 6 or 30 sterile units (Cu-7 with an inserter) and sufficient patient identification cards.

Clinical Studies: Different event rates have been recorded with the use of different intrauterine contraceptive devices. Inasmuch as these rates are usually derived from separate studies conducted by different investigators in several population groups, they cannot be compared with precision. Furthermore, event rates tend to be lower as clinical experience is expanded, possibly due to retention in the clinical study of those patients who accept the treatment regimen and did not discontinue due to adverse reactions or pregnancy. In clinical trials of the Cu-7 conducted by Searle Laboratories, use effectiveness was determined as follows for parous and nulliparous women, as tabulated by the life table method. (Rates are expressed as events per 100 women through 12 and 24 months of use.) This experience encompasses 305,958 woman-months of use, including 12 months for 9,549 women and 24 months for 7,425.

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	Parous	Nulliparous	Parous	Nulliparous
Pregnancy	1.6	1.2	2.4	2.0
Expulsion	5.4	7.7	6.4	8.7
Medical Removal	9.3	12.3	14.9	17.2
Continuation Rate	78.7	73.2	65.0	60.5

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1. Bonnar, J., et al., Lancet, 1:457, 1969.
2. Porter, A. M. W., Brit. Med. J., 1:218, 1969.

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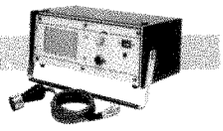
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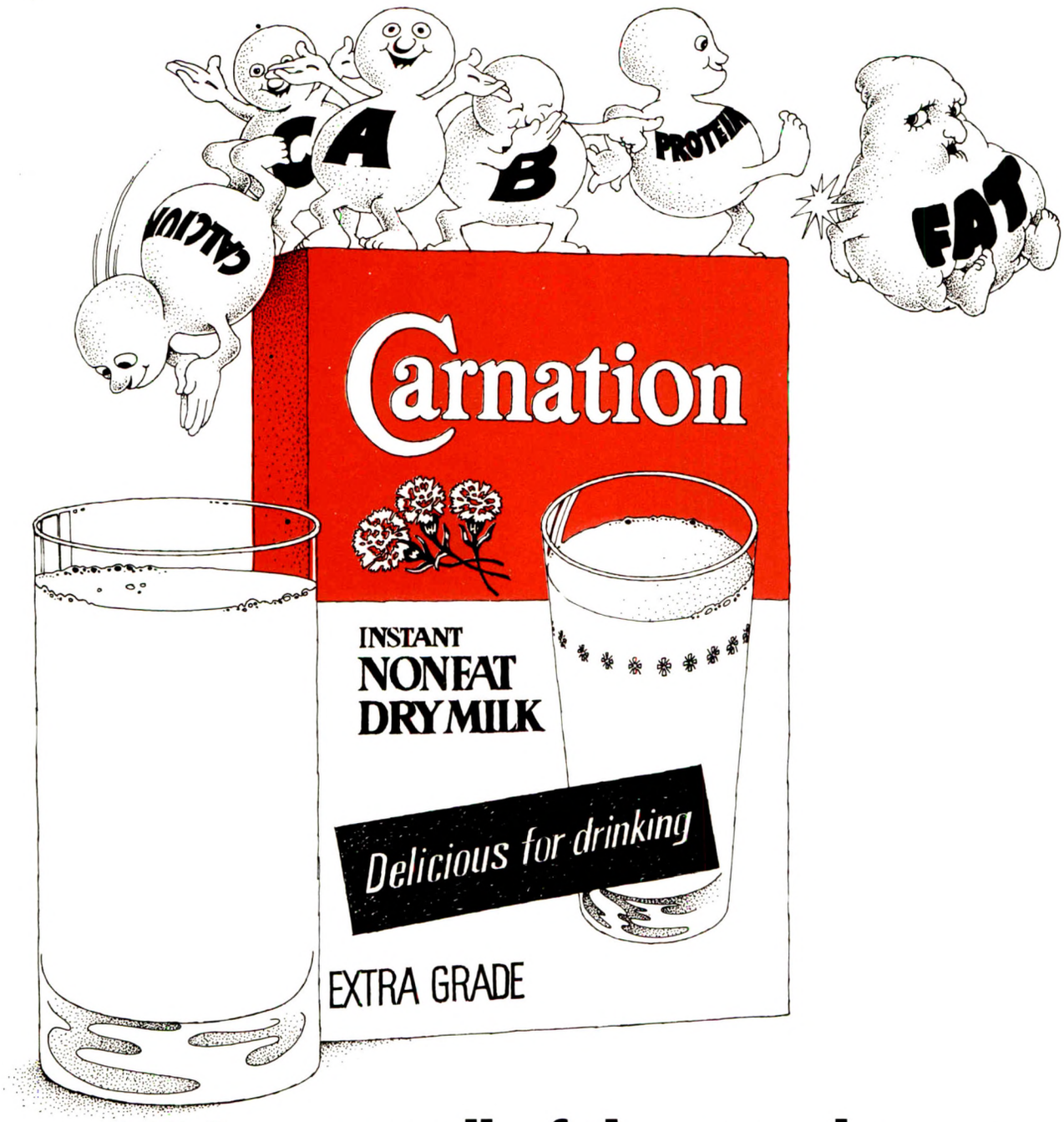
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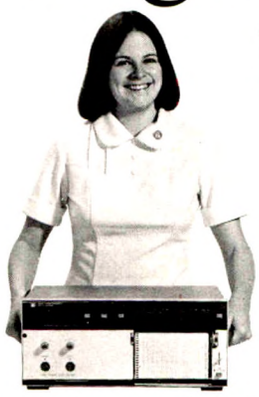
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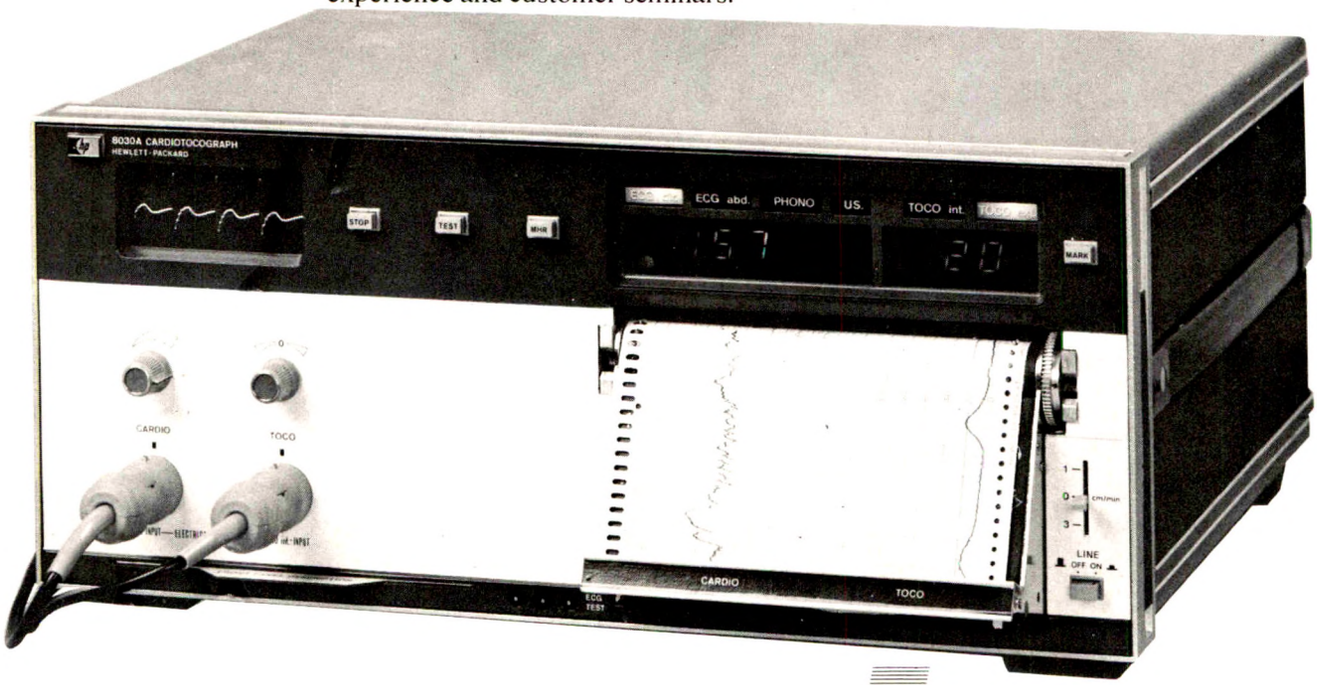
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Contraindication Patients known to be hypersensitive to this drug.

Precautions *General:* Discontinue drug if sensitization or irritation is reported during use. *Laboratory Tests:* If there is a lack of response to MONISTAT, appropriate microbiological studies should be repeated to confirm the diagnosis and rule out other pathogens. *Pregnancy:* MONISTAT Cream was used in 209 pregnant patients. Follow-up reports now available in 117 of these patients reveal no adverse effects or complications attributable to MONISTAT therapy in infants born to these women. Since MONISTAT Cream is absorbed in small amounts from the human vagina, it should be used in the first trimester of pregnancy only when the physician considers it essential to the welfare of the patient.

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Dosage and Administration One applicatorful is administered intravaginally once daily at bedtime for 14 days. Course of therapy may be repeated after other pathogens have been ruled out by appropriate smears and cultures.

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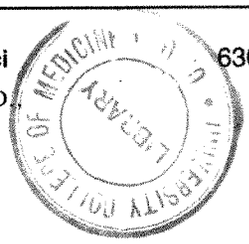
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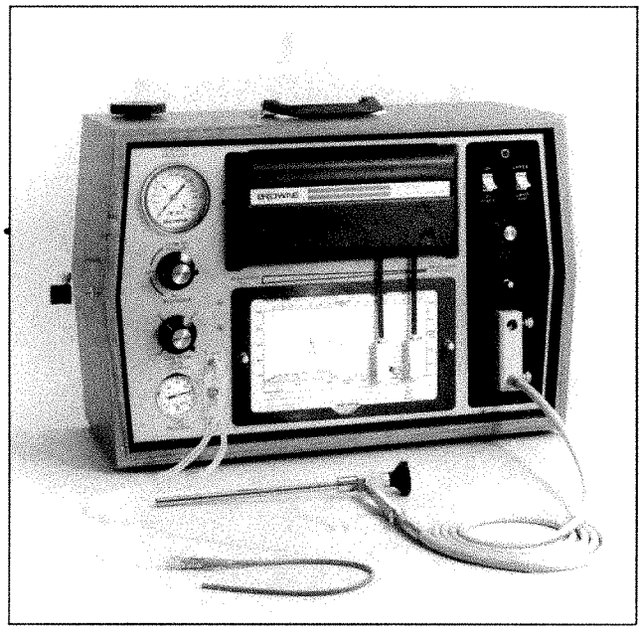
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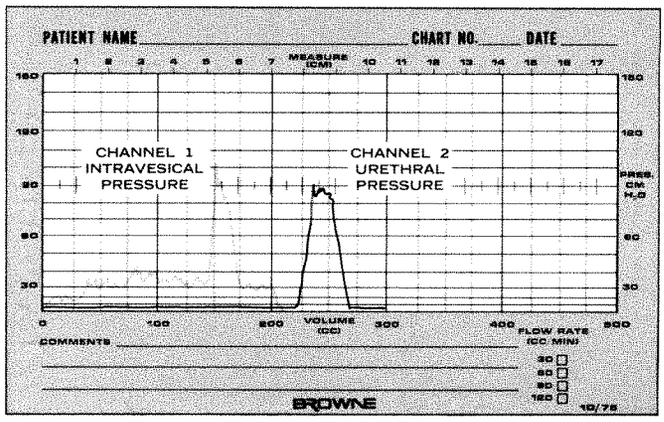
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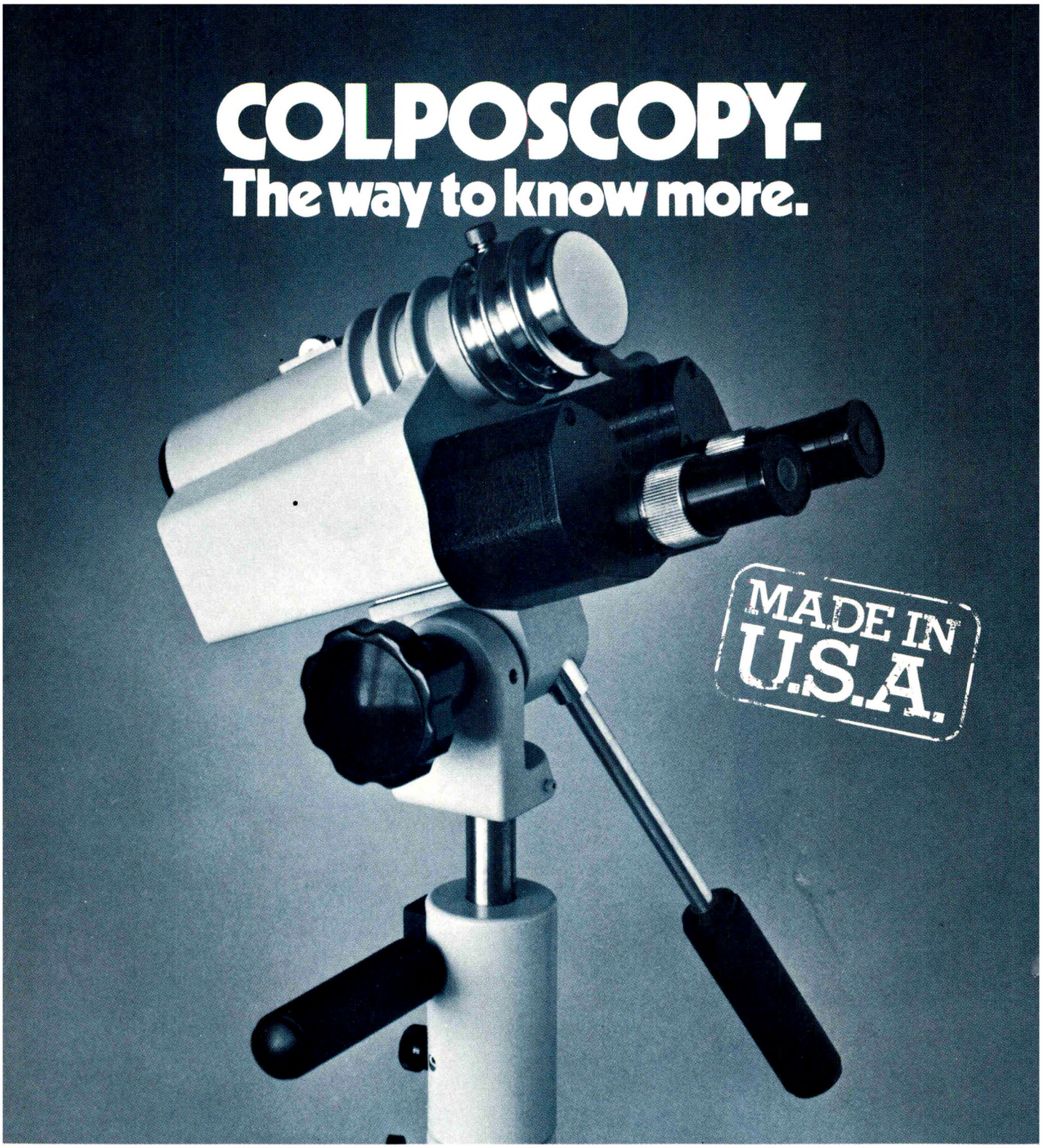
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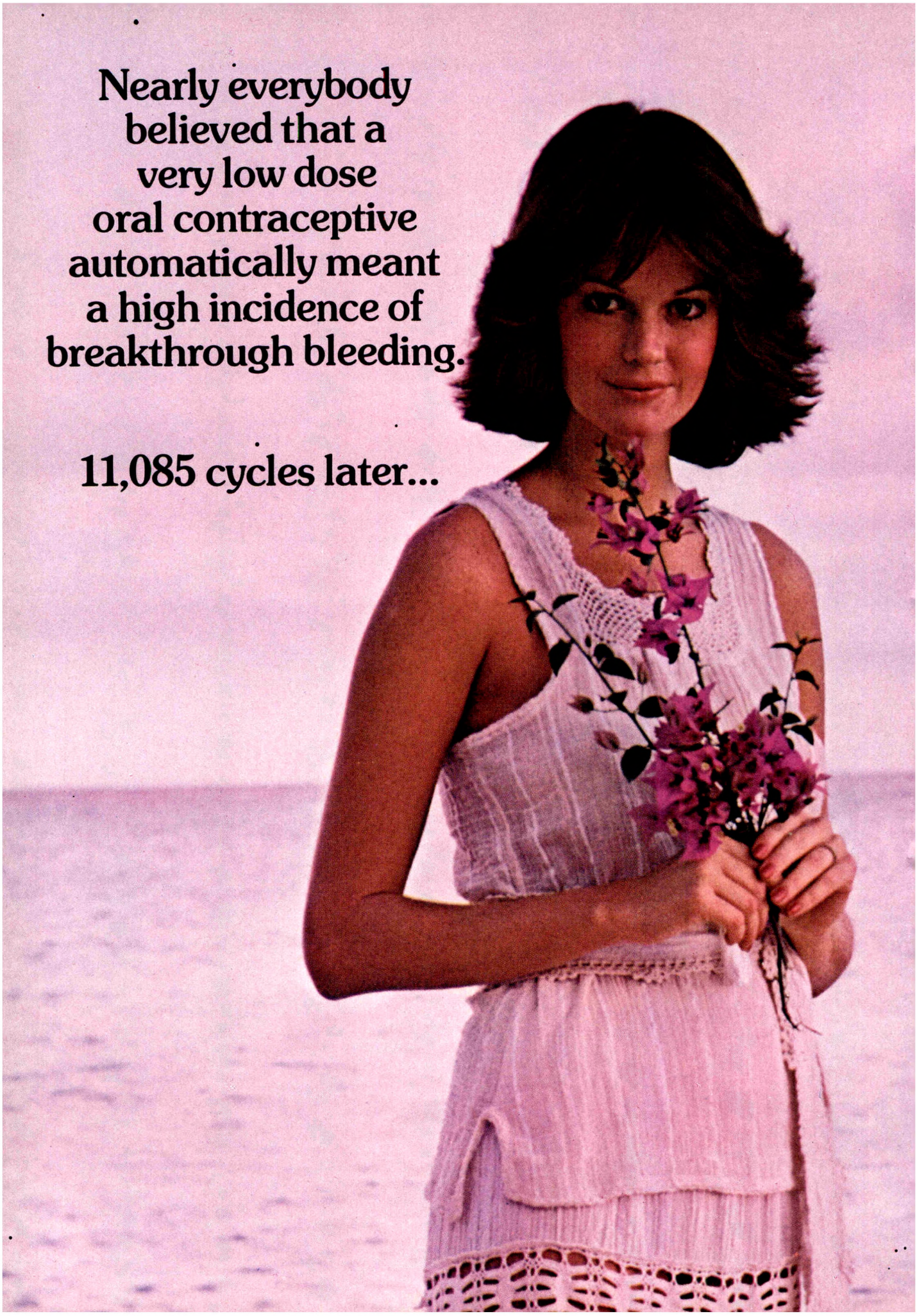
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Incidence of Breakthrough Bleeding in Premarketing Clinical Trials

Cycle	1	3	6	12	Total Cycles
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Breakthrough Bleeding	9.0%	3.5%	2.7%	2.6%	3.9%

Acceptance has been excellent

The low incidence of minor side effects has resulted in ready acceptance of LO/OVRAL by the great majority of patients. Patient complaints have been infrequent. Only eight percent of women participating in clinical trials discontinued LO/OVRAL because of common side effects.

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The dosage regimen with LO/OVRAL is convenient and easy to follow: 3 weeks on, 1 week off. The attractive, uncomplicated Pilpak® dispenser helps patients follow the right dosage schedule.

*Serious as well as minor adverse reactions have been reported following the use of all oral contraceptives. The physician should remain alert to their earliest manifestations and in the event of serious adverse reactions should avoid any oral contraceptives. *Special note, contraindications, warnings, precautions and adverse reactions* in the accompanying IN BRIEF should be carefully considered.

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Important information on next page.

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Low dose oral contraceptive.

IN BRIEF:

SPECIAL NOTE: Oral contraceptives have been marketed in the United States since 1960. Reported pregnancy rates vary from product to product. The effectiveness of the sequential products appears to be somewhat lower than that of the combination products. Both types provide almost completely effective contraception.

An increased risk of thrombotic, thromboembolic and cerebral-vascular disease associated with the use of hormonal contraceptives has been shown in studies conducted in both Great Britain and the United States. Other risks, such as those of elevated blood pressure and reduced tolerance to carbohydrates, have been reported, and appropriate tests should be conducted to monitor these during oral-contraceptive therapy. Liver disease has also been reported, and the physician should be alert to its earliest manifestations.

Long-term administration of both natural and synthetic estrogens in subprimate animal species in multiples of the human dose increases the frequency for some animal carcinomas. These data cannot be transposed directly to man. The possible carcinogenicity due to the estrogens can neither be affirmed nor refuted at this time. Close clinical surveillance of all women taking oral contraceptives must be continued.

INDICATIONS: For oral contraception.

CONTRAINDICATIONS: 1. Thrombophlebitis, thromboembolic disorders, cerebral-vascular disease, or a past history of these conditions; 2. Markedly impaired liver function; 3. Known or suspected carcinoma of the breast; 4. Known or suspected estrogen-dependent neoplasia; 5. Undiagnosed abnormal genital bleeding; 6. Known or suspected pregnancy.

WARNINGS: 1. The physician should be alert to the earliest manifestations of thrombotic and thromboembolic disorders, thrombophlebitis, cerebrovascular disorders including hemorrhage, pulmonary embolism and retinal thrombosis. Should any of these occur or be suspected, the drug should be discontinued immediately.

Retrospective studies of morbidity and mortality in Great Britain and studies of morbidity in the United States have shown a statistically significant association between thrombophlebitis, pulmonary embolism and cerebral-vascular disease, occlusive or hemorrhagic, and the use of oral contraceptives. There have been three principal studies in Great Britain[1-3] leading to these conclusions and three[4-7] in this country. The estimate of the relative risk of thromboembolism in the study by Vessey and Doll[3] was about sevenfold, while the United States studies found relative risks of 4.4 to 11, meaning that the users are several times as likely to undergo thromboembolic disease without evident cause as non-users.

In May, 1974, the Royal College of General Practitioners in England[8] issued an interim report of its continuing large-scale prospective study comparing a user group to a non-user group. This study in its interim analysis states: "A statistically significant higher rate of reporting of cerebrovascular accidents in Takers is evident, but the numbers are too small to justify an estimation of the degree of risk." The study also reported a higher incidence of superficial and deep vein thrombosis in users as compared to non-users.

The Sartwell study[4] indicated that the risk did not persist after discontinuation of administration. Both the Sartwell and the Royal College study indicated that the degree of risk was not associated with duration of treatment.

In a collaborative American study[5,6] of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the relative risk of thrombotic stroke was 4.1 to 9.5 times greater in users than in non-users. A comparable estimate for hemorrhagic stroke was 2.0.

None of the American studies was designed to evaluate a difference between products. However, the Sartwell study[4] suggested that there might be an increased risk of thromboembolic disease in users of sequential products.

Other retrospective studies[9,10] have reported an increased risk of post-surgery thromboembolic complications in oral-contraceptive users. It has been recommended that therapy be discontinued at least one month prior to elective surgery.

2. Discontinue oral-contraceptive medication if there is: gradual or sudden, partial or complete loss of vision; proptosis or diplopia; onset or aggravation of migraine or development of headache of a new pattern which is recurrent, persistent or severe; papilledema; or any evidence of retinal-vascular lesions. 3. Fetal abnormalities have been reported to occur in the offspring of women who have taken progestogens and/or estrogens during pregnancy.[11,12] The safety of LO/OVRAL® in pregnancy has not been demonstrated. Pregnancy should be ruled out before initiating or continuing the contraceptive regimen. Pregnancy should always be considered if withdrawal bleeding does not occur. 4. A small fraction of the hormonal agent in oral contraceptives has been identified in the milk of mothers receiving these drugs. The long-range effect to the nursing infant cannot be determined at this time. 5. Hepatic lesions (adenomas, hepatomas, hamartomas, regenerating nodules, etc.), occasionally fatal, have been reported in women on oral contraceptives. Such lesions may present as an abdominal mass or with the signs and symptoms of an acute abdomen. These lesions should be considered if the patient has abdominal pain or evidence of intra-abdominal bleeding. Such lesions have been reported in short-term as well as long-term users of oral contraceptives.

PRECAUTIONS: 1. A thorough history and physical examination should be performed before prescribing oral contraceptives and periodically during their administration and should include special reference to breasts and pelvic organs including Papanicolaou smears, since estrogens have been known to produce tumors, some of them malignant, in five species of subprimate animals. 2. Endocrine and possibly liver-function tests may be affected by treatment with LO/OVRAL® Therefore, if such tests are abnormal in a patient taking LO/OVRAL® it is recommended that they be repeated after the drug has been withdrawn for two months. 3. Under the influence of estrogen-progestogen preparations, pre-existing uterine fibromyomata may increase in size. 4. Because these agents may cause some degree of fluid retention, conditions which might be influenced by this factor, such as epilepsy, migraine, asthma, cardiac or renal dysfunction, require careful observation. 5. In breakthrough bleeding, and in all cases of irregular bleeding per vaginam, nonfunctional causes should be borne in mind. In undiagnosed bleeding per vaginam, adequate diagnostic measures are indicated. 6. Patients with a history of psychic depression should be carefully observed and the drug discontinued if the depression recurs to a serious degree. 7. Any possible influence of prolonged LO/OVRAL® therapy on pituitary, ovarian, adrenal, hepatic or uterine function awaits further study. 8. A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. The mechanism of this decrease is obscure. For this reason, diabetic patients should be carefully observed while receiving LO/OVRAL® therapy. 9. The age of the patient constitutes no absolute limiting factor, although treatment with LO/OVRAL® may mask the onset of the climacteric. 10. The pathologist should be advised of LO/OVRAL® therapy when relevant specimens are submitted. 11. Susceptible women may experience an increase in blood pressure following administration of contraceptive steroids. 12. Cholestatic jaundice has been reported in users of oral contraceptives. If this occurs, LO/OVRAL® should be discontinued. This condition is more likely to occur in patients who have experienced cholestatic jaundice of pregnancy. Patients with a history of cholestatic jaundice of pregnancy should be carefully observed during LO/OVRAL® therapy.

ADVERSE REACTIONS OBSERVED IN PATIENTS RECEIVING ORAL CONTRACEPTIVES: A statistically significant association has been demonstrated between use of oral contraceptives and the following serious adverse reactions: thrombophlebitis, pulmonary embolism, cerebral thrombosis and hemorrhage, gall bladder disease.

Although available evidence is suggestive of an association, such a relationship has been neither confirmed nor refuted for the following serious adverse reactions: neuro-ocular lesions, e.g., retinal thrombosis, optic neuritis and hepatic lesions with or without intra-abdominal bleeding.

The following adverse reactions are known to occur in patients receiving oral contraceptives: nausea, vomiting, gastrointestinal symptoms (such as abdominal cramps and bloating), breakthrough bleeding, spotting, change in menstrual flow, amenorrhea during and after treatment, edema, chloasma or melasma, which may persist, breast changes: tenderness, enlargement and secretion, change in weight (increase or decrease), changes in cervical erosion and cervical secretions, suppression of lactation when given immediately post-partum, cholestatic jaundice, migraine, rash (allergic), rise in blood pressure in susceptible individuals, mental depression.

Although the following adverse reactions have been reported in users of oral contraceptives, an association has been neither confirmed nor refuted: anovulation post treatment, which tends to occur more frequently in women with a history of menstrual disorders, premenstrual-like syndrome, changes in libido, changes in appetite, cystitis-like syndrome, headache, intolerance to contact lenses, nervousness, dizziness, fatigue, backache, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, itching and vaginitis.

The following laboratory results may be altered by the use of oral contraceptives: hepatic function; increased sulfobromophthalein retention and other tests; coagulation tests; increase in prothrombin, Factors VII, VIII, IX and X; decrease in antithrombin III; increase in platelet aggregability; thyroid function increase in PBI, and butanol extractable protein-bound iodine and decrease in T^3 uptake values; metyrapone test; and pregnanediol determination.

NOTE: For more specific details on LO/OVRAL, refer to package insert.

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CLINICAL OPINION

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FDA obstetrics-gynecology device classification report

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The Panel on Review of Obstetrical and Gynecological Devices of the Food and Drug Administration has tentatively recommended that all diagnostic, surgical, monitoring, therapeutic, and prosthetic devices in current use in the discipline be designated according to perceived need for scientific review, specific standards, and/or general controls for purposes of regulatory classification. Feedback is solicited from all obstetrician-gynecologists and other concerned citizens.

THE BUREAU of Medical Devices and Diagnostic Products of the Food and Drug Administration, through its Panel on Review of Obstetrical and Gynecological Devices, has been involved in fulfilling its charge to classify devices in use in our discipline in anticipation of regulatory legislation aimed at protecting the public. We wish to make known our tentative decisions on the assignment of such devices into

From the Food and Drug Administration.
Reprint requests: Emanuel A. Friedman, M.D., 330 Brookline Ave., Boston, Massachusetts 02215.

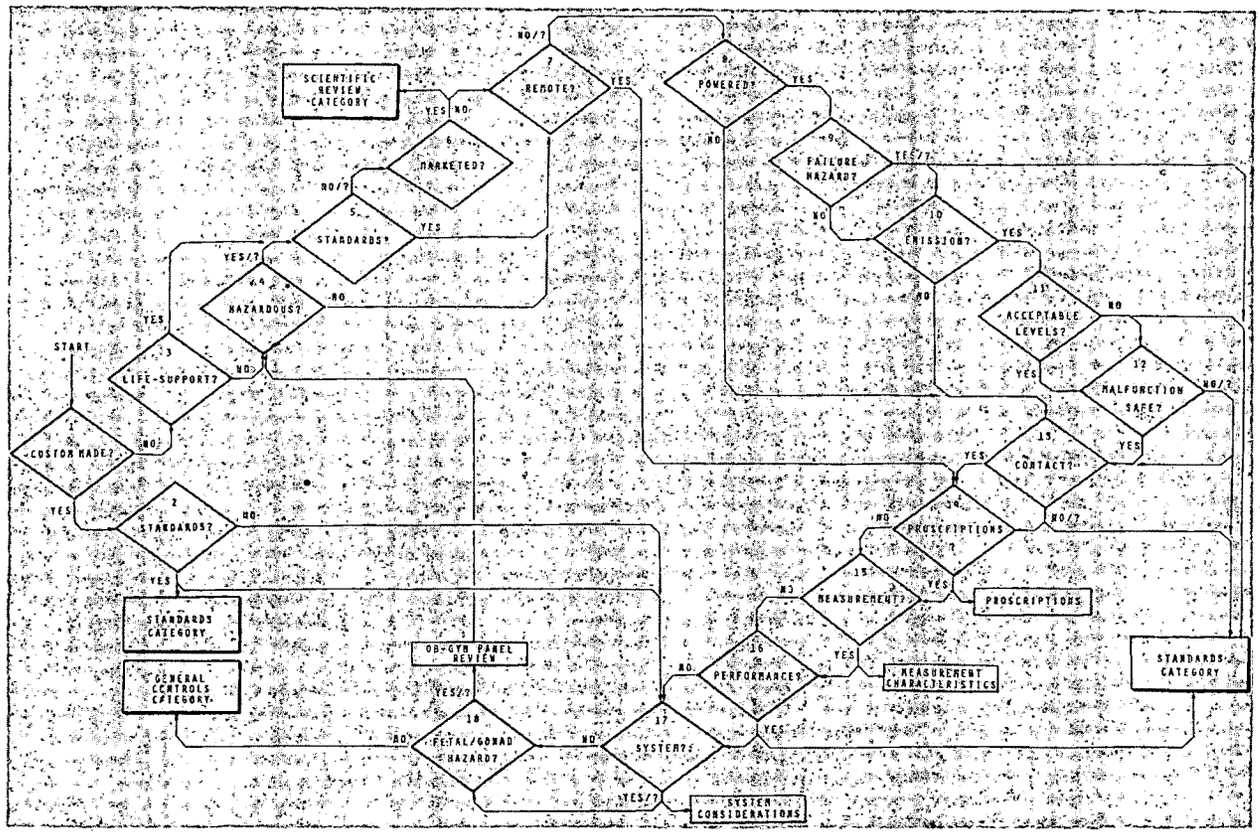


Fig. 1. Schematic flow diagram of the logic system for classifying medical devices into regulatory categories. See text for details of the questions posed and the significance of the categories.

appropriate regulatory categories for both the information of and review and comment by all obstetricians and gynecologists.

In perspective, the need for medical device legislation was brought into national focus in 1969 by a presidential address to Congress on "Protection of Interests of Consumers." As a consequence of this statement, the Department of Health, Education and Welfare, under the direction of the Assistant Secretary for Health and Scientific Affairs, appointed a study committee chaired by Dr. Theodore Cooper, Director of the National Heart and Lung Institute, National Institutes of Health. The committee was asked to provide the framework for medical device legislation, concentrating particularly on procedures for the establishment of medical device standards and for the premarketing review and regulation of devices.

The committee's report, first made public in September, 1970, recommended that scientific, health, and engineering experts be assembled to review existing devices and to advise on their classification into three groups: (1) a general control category, including those devices that should be exempt from standard-setting and premarketing review; (2) a standards category, encompassing those devices that required the formulation and maintenance of standards in order to assure safety and efficacy; and (3) a scientific review category, to specify those which needed premarketing clearance.

Devices falling into the general controls category must satisfy requirements relating to manufacturer registration, record and report keeping, notification of defects and repair or replacement, conformity with good manufacturing practices, factory inspection, and adequate labeling for safe and effective use. Devices placed in the standards category, on the other hand, have safety and performance characteristics that are best controlled by the establishment of standards that can be agreed upon by the scientific and medical community based on existing data. The standards include those of electrical safety, material characteristics, biocompatibility, energy-emission levels, and device performance, and will be developed in due course.

For a device to be classified as requiring scientific review, it must be life-supporting, life-sustaining, or potentially hazardous to life or good health. In addition it must be one for which sufficient scientific and medical data are not presently available to permit

establishing adequate standards to control safety and efficacy. Alternatively, a device is placed in this category if the development and application of such standards would not be adequate for control purposes. Scientific review requires premarketing assessment by an outside body of experts until such time as evidence can be provided to support the adoption of adequate standards for a given device. When such standards are developed, the device may then be reclassified accordingly.

Based on these recommendations, 14 specialty-oriented panels of experts were established by the Food and Drug Administration beginning in 1971. These include (in order of creation): orthopedics, cardiovascular, dental, anesthesia, obstetrics and gynecology, gastroenterology and urology, diagnostic products, general hospital and personal use devices, neurology, otolaryngology, plastic and general surgery, ophthalmology, physical medicine, and radiology panels.

Each of the panels was charged with (1) classification of all devices in use of the specialty, (2) identification of specific known device hazards and problems, (3) recommendations concerning those device characteristics for which standards should be developed, and (4) preparation of guidelines for product development protocols. The membership of the panels includes both clinicians and researchers, as well as consumer and industry liaisons, together with a representative from the Division of Classification and Scientific Evaluation of the Bureau of Medical Devices and Diagnostic Products who serves as executive secretary.

A classification logic scheme was evolved by the first two pilot panels for purposes of dealing with use, safety, risk, and properties of each device in an organized, consistent, objective, and systematic fashion. The logic system was adopted for use uniformly by all panels. The Panel on Review of Obstetrical and Gynecological Devices, shortly after it was formed in 1973, recommended a mechanism for routine consideration of every device in all panels as to its potential fetal and genetic hazards. This was accomplished by modifying the original classification logic system so that a specific query in this regard would be posed for each device being classified.

Pending legislation before the Congress proposes the regulatory categories that have been identified in the classification system. It is clear that, even without such a law, classification of devices should prove useful for purposes of identifying devices of a critical nature as well as elucidating standards of safety and performance that should be met. Needless to say, the three categories are not mutually exclusive, so that a device may be assigned to one or more of them. Moreover, it is understood that the classification of any given device may be subject to change if it is determined to require reclassification through newly acquired information.

The classification logic system has been applied to each device to determine which of the control classes is specifically appropriate to it. Inherent in this system is the consideration of the concept of the relationship between benefit and risk. The sequence of questions leads one to the appropriate control categories according to the pathways shown diagrammatically in Fig. 1. The specific questions (edited for clarity) are as follows.

1. Is the device custom made?
2. Although the device is custom made, can standards be applied?
3. Is the device life-sustaining or life-supporting?
4. Is the device or the mechanism by which the diagnostic information is derived potentially hazardous to life or good health when properly used?
5. Is the device of such a nature that (1) sufficient scientific and medical data exist from which adequate standards governing its safety and efficacy could now be established, and (2) the development and application of such standards would be adequate to control the device?
6. Is the device currently in use and marketed in the United States?
7. When the device is used, is it remote from the body?
8. Is the device powered by a nonmanual external or internal source?
9. Will use of the device or failure of power or failure of the device power source present a potential hazard to the patient?
10. Does the device emit or inject any form of energy into the body?
11. Have the energy levels used been shown to be acceptable?
12. Will malfunction of the device result in safe energy levels?
13. Does the device use material for contact with the body which is generally acceptable or has known and acceptable properties requiring no additional controls?
14. Does the device have any known hazards, limitations, or shortcomings which can be avoided by the promulgation of federal regulations?
15. If the device performs some measurement function, should the accuracy, reproducibility, or limitations of the information supplied be clearly indicated to the user by appropriate labeling, instructions, or cautions?
16. Does the device have performance characteristics which should be maintained at a satisfactory level, such

Table I. Regulatory classification of obstetrics-gynecology devices

A. Scientific review category:*
- Diagnostic devices
  - Hysteroscopes and distending media (as a system)
- Monitoring devices
  - Arterial pressure monitors, ultrasonic
  - Blood flow monitors, ultrasonic
  - Fetal head diameter measurement instruments, ultrasonic
  - Fetal imagers, ultrasonic
  - Heart rate monitors, ultrasonic
  - Heart sound monitors, ultrasonic
  - Heart valve movement monitors, ultrasonic
  - Hemic sound monitors, ultrasonic
  - IUD locators, ultrasonic
  - Perinatal monitoring complexes
  - Ultrasonic transducers
- Surgical devices
  - Hysteroscopic coagulators and accessories
  - Laser-powered coagulators and accessories
  - Metreurynter balloon abortion units
  - Tubal occlusion bands
  - Tubal occlusion clips
  - Tubal occlusion inserters
  - Tubal occlusion valves
  - Hygroscopic cervical dilators, laminaria
  - Vibratory cervical dilators
- Therapeutic devices
  - Intrauterine contraceptive devices
  - IUD introducers
  - Abdominal decompression chambers

B. Standards category†:
- Diagnostic devices
  - Amnioscopes, transabdominal (8)‡
  - Amnioscopes, transcervical (16)
  - Colpomicroscopes
  - Colposcopes
  - Culdoscopes (7)
  - Laparoscopes (3)
  - Uterotubal CO_2 insufflators (9)
  - Endometrial aspirator bulbs and pipettes (27)
  - Endocervical aspirators
  - Endometrial brushes (18)
  - Cervical cytology kits
  - Endometrial suction biopsy curettes (17)
  - Gynecologic biopsy forceps
  - Cervical cytology spatulas
  - Endometrial washers (15)
- Monitoring devices
  - Fetal status data analyzers
  - Fetal scalp blood sampling endoscopes and obturators (6)
  - Fetal scalp blood sampling kits (6)
  - Fetal scalp electrodes (11)
  - Fetal scalp clip applicator
  - Fetal cardiotachometers
  - Fetal electrocardiographic monitors
  - Fetal electroencephalographic monitors
  - Fetal phonocardiographic monitors
  - Intrauterine catheters (5)

*Includes standards and general regulatory categories as well.

†Includes general controls also.

‡Order of priority indicated in parentheses for all devices that carry significant hazard in clinical use and have been assigned to standards category.

Table I—Cont'd

B. Standards category†:—continued
- Intrauterine catheter introducers (5)
- Intrauterine pressure recorders
- Intrauterine pressure transducers
- Tocodynamometers
- Prosthetic devices
  - Cervical drains (29)
  - Vaginal molds
  - Fallopian tube prostheses (28)
- Surgical devices
  - Culdoscopic coagulators and accessories (2)
  - Laparoscopic coagulators and accessories (2)
  - Cervical cryosurgical instruments and accessories (12)
  - Cervical electrocautery instruments and accessories (13)
  - Uterine suction cannula (1)
  - Vacuum abortion units (1)
  - On-off suction controllers (1)
  - Vacuum sources (1)
  - Amniotomes
  - Vaginal applicator
  - Uterine insufflation cannulas and accessories
  - Circumcision clamps
  - Umbilical clamps
  - Uterine clamps
  - Cleidoclasts
  - Cranioclasts
  - Craniotribes
  - Uterine curettes
  - Expandable cervical dilators
  - Fixed-size cervical dilators
  - Vaginal dilators
  - Uterine elevators
  - Vacuum extractor (10)
  - Surgical forceps and accessories
  - Obstetric forceps and accessories
  - Fibroid hooks
  - Destructive obstetric hooks
  - Cervical conization knives
  - Cerclage needles
  - Uterine packers
  - Vaginal retractors
  - Episiotomy scissors
  - Umbilical scissors
  - Fibroid screws
  - Uterine sounds
  - Vaginal specula, illuminated
  - Vaginal specula, nonilluminated
  - Obstetric delivery tables and accessories (20)
  - Obstetric delivery tables, powered (19)
  - Uterine tenacula
- Therapeutic devices
  - Diaphragms (26)
  - Diaphragm introducers
  - IUD hook-type removers (25)
  - Cervical caps
  - Menstrual cups
  - Vaginal insufflators (23)
  - Vaginal douche kits (23)
  - Menstrual pads
  - Vaginal pessaries
  - Sitz bath kits
  - Tampons
  - Paracervical anesthesia sets (4)
  - Pudendal anesthesia sets (14)
  - Abdominal binders
  - Breast binders

Table I—Cont'd

B. Standards category†:—continued
- Maternity and nursing brassieres
- Maternity girdles
- Perineal heating devices, direct contact (21)
- Perineal heating devices, radiant (22)
- Breast pads
- Breast pumps, manual
- Breast pumps, powered (24)
- Breast shields

C. General control category (only):
- Diagnostic devices
  - Pelvimeters
- Monitoring devices
  - Fetoscopes
- Surgical devices
  - Drape clips

level having general agreement among the user groups?

17. Is the device used with other devices in such a way that the system in which it is used can be hazardous if the system is not assembled, used, or maintained in a satisfactory fashion?

18. Is the device potentially hazardous to the fetus or the gonads when properly used?

It should be noted that special attention is directed toward the objective of providing proscriptions (question 14) for those devices or classes of devices that are shown to have limitations, hazards, difficulties, or problems which can be controlled by specific regulations. It is expected that such proscriptions will become an inherent part of the labeling of such devices for purposes of ensuring limited use. For devices performing measurement functions (question 15), measurement characteristics, particularly as they refer to performance, accuracy, or use considerations, will also be clearly indicated in the labeling so that this information will be readily available to the user. Systems considerations, to which question 17 is directed, would provide the application of controls currently beyond the jurisdiction of the current federal Food, Drug and Cosmetic Act, but will be encouraged through the intermedium of responsible, interested organizations both in and out of government with more jurisdiction and authority. Devices known to be potentially hazardous to the fetus or the gonads, or for which such information is unknown (question 18), are to be reviewed by the Panel on Review of Obstetrical and Gynecological Devices amd the original classifying panels jointly for purposes of adjudication and, if necessary, reclassification.

The Panel on Review of Obstetrical and Gynecological Devices has thus far considered in detail 122 devices and classes of devices representative of the full gamut of devices in use in the field. Classification has proceeded in an orderly manner, the list of devices having first been presorted into five usage groupings: (1) 17 diagnostic devices, (2) 26 monitoring devices, (3) three prosthetic devices, (4) 50 surgical devices, and (5) 26 therapeutic devices. Table I presents the entire list with the tentative assignment to regulatory classification as derived by the logic system described above.

General controls were applicable to all devices, but in three instances no other form of regulatory control was necessary. Some form of standards was required for all others. The panel held that premarketing scientific review was essential for 24 devices. These latter included nine groups of devices (in order of priority): (1) intrauterine contraceptive devices and introducers, (2) diagnostic ultrasonic devices, (3) hysteroscopes and distending media (as a system), (4) cervical vibratory dilators, (5) laser and hysteroscopic coagulation units and accessories, (6) hygroscopic cervical dilators (laminaria), (7) tubal occlusion bands, clips, valves, and inserters, (8) metreurynter balloon abortion units, and (9) abdominal decompression chambers.

The panel is now considering specific device hazards that require scientific review as well as device characteristics that demand the formulation of standards. The members of the panel feel that they can be of greatest assistance by recommending specific guidelines for scientific review procedures. We are requesting that information be transmitted by all practitioners in the specialty concerning clinical problems with the devices and with specific characteristics of the devices for which standards should be developed. Through the Bureau of Medical Devices and Diagnostic Products, data and suggestions will be collected from various standards committees, scientific and engineering groups, industry, the medical profession, and all interested parties. Such information will be studied in detail and used for purposes of recommending guidelines for scientific review procedures and mechanisms for the development of appropriate standards.

Each of the meetings of the panels, with agenda details, is announced well in advance by publication in the Federal Register. All concerned citizens, whether professional, industrial, or lay representatives, are invited to attend and to participate. Comments regarding clinical problems with devices under consideration and suggestions for safety and performance standards should be directed to Lillian Yin, Ph.D., Executive Secretary, Bureau of Medical Devices and Diagnostic Products (HFK-400), Food and Drug Administration, 5600 Fishers Lane, Rockville, Maryland 20852. Your responses are earnestly solicited.

GYNECOLOGY

Vascular injuries in pelvic cancer surgery

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Although pelvic cancer surgery is attended by many difficulties, vascular injuries are actually rare. New techniques of isotopic and ultrasound monitoring allow objective assessment of such patients with vascular injury. In venous problems, absolutely precise diagnosis is now possible and in both arterial and venous injury, extra-anatomic reconstructions can be done which return the vascular status of the patient to near normal.

THOUGH ARTERIAL and venous injuries in pelvic cancer surgery are rare, they do occur. As interest increases in radical removal of pelvic and inguinal lymph nodes in association with ablation of genital cancer, it is to be expected that iatrogenic injury of major vascular structures will continue.

Vascular injuries associated with extirpation of pelvic malignancies can be classified readily into arterial and/or venous injury. While arterial injury takes many forms, with acute and chronic manifestations, venous injury results in occlusion by thrombosis or by transection.

Hemorrhage is a complication which is common to both arterial and venous injury, but is more dangerous and difficult to control when from the low-pressure venous system. Conventional weapons of attack upon unsuspected surgical bleeding are the pack and the suture. However, extensive experience in anticoagulated cardiovascular surgical patients and in those with inherent coagulopathy has shown that the electrocautery greatly aids in maintaining operative control. General surgery is following the lead of vascular and transplant surgeons who have proven the utility and safety of the electrocoagulator.

Electrocautery is not commonly used in gynecology and many pelvic surgeons are not familiar with its use. Nevertheless, in other surgical fields, some of the most difficult surgical procedures are rendered easier by electrocoagulation. Perhaps this tool should become a regular part of the armamentarium of the gynecologic oncologist.

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Arterial injury

Chronic forms of arterial injury such as false aneurysm formation or arteriovenous fistula allow sufficient time for contemplation, diagnosis, and consultation and will not be discussed here (Fig. 1). Principles of treatment of such lesions merely involve restoration of arterial continuity and preservation of venous flow wherever possible. The basic tenet of autogenous tissue repair dominates judgment in such situations.

Acute arterial injuries do not allow time for contemplation. They must be managed effectively immediately. Simple laceration of the artery wall or total, through-and-through penetrations are best treated by

direct suture with fine, interrupted, nonabsorbable, vascular suture material. Partial loss of the arterial wall is more serious and requires repair with autogenous tissue patching. The best tissue available for this is saphenous vein, which can be removed from the ankle or the groin. One should not use more easily accessible abdominal veins such as the ovarian because these will not withstand arterial pressure.

While mobilizing the injured artery and during actual clamping, the patient must be systemically heparinized. This can be accomplished with 5,000 units of intravenous heparin and then neutralized with a like amount of protamine sulfate once the arterial flow has been restored.

Situations which produce arterial occlusion include complete transection, adventitial hematoma, and intimal fracture. These resemble myogenic spasm but do not respond to conservative local vasodilation. If myogenic spasm is seen at the operating table or is suspected during the course of periadventitial dissection, one can relieve such spasm by bathing the affected vascular structures with a solution of 60 mg. of papaverine in 100 ml. of saline.

The lesions of complete transection, adventitial hematoma, and intimal fracture are all three managed best by wide exposure of the affected vessel in its area of occlusion, atraumatic occlusion of the vessel itself, and direct arteriotomy with specific reconstruction. In repairing direct transection, mobilization, debridement of arterial edges, and direct suture is sufficient. However, intimal fracture and subadventitial hematoma are best managed by arteriotomy and direct vision repair, followed by a vein patch closure of the arterial incision.

Arterial monitoring

It is of greatest importance to the gynecologic oncologist that he appreciate the fact that noninvasive techniques are now available to monitor patients in the recovery room so that early detection of arterial occlusion can be achieved. In response to a need for precision in evaluation of vascular surgical patients with arterial repair, instrumentation has been developed and is achieving wide acceptance. Among these methods, the ultrasound technique has emerged as being the most portable and accurate.[1]

At the Northwestern University Medical Center, infection and injury to the external iliac artery following pelvic ablative surgery was formerly treated by ligation of the artery proximal to the infection and bypass from the nonaffected common femoral artery to the femoral artery on the side of the lesion. This type of bypass was performed in order to maintain

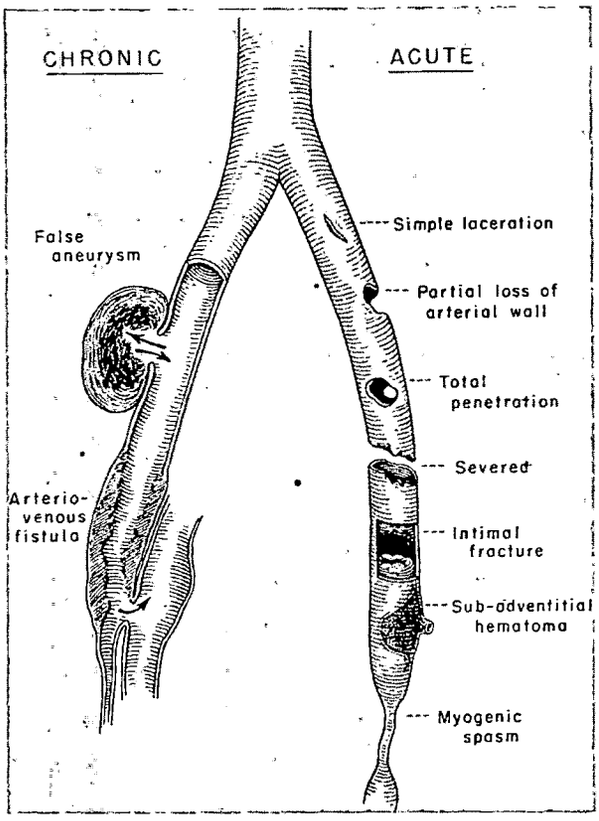


Fig. 1. Arterial injuries caused by surgery for pelvic malignancy may result in acute or chronic problems, as illustrated here. (From Jackson, B. B.: Surgery of Acquired Vascular Disorders, Springfield, Ill., 1969. Courtesy of Charles C Thomas, Publisher.)

arterial continuity despite arterial ligature performed in dealing with the abscess of the arterial wall.

Since the advent of the ultrasound technique, it has been possible to monitor the patient's ankle arterial pressure during proximal arterial ligation and, by such monitoring, determine those patients not needing arterial reconstruction. It is known that a limb will survive anatomically if the ankle blood prssure can be maintained at a ratio of 0.3 to brachial blood pressure. Such measurement and evaluation can easily be performed by a technician or nurse in the recovery room following iliac artery ligation.[2] A fall in ankle pressure below this level indicates that the patient must be returned to the operating room for a cross-over arterial reconstruction.

Therefore, at present, injury is suspected in the pelvis, ultrasound ankle pressures are obtained and recorded. If the ankle pressure is one half that of the brachial blood pressure, anticoagulation is begun. If the pressure falls below 0.3, plans are made for retrograde femoral arteriography and direct repair of

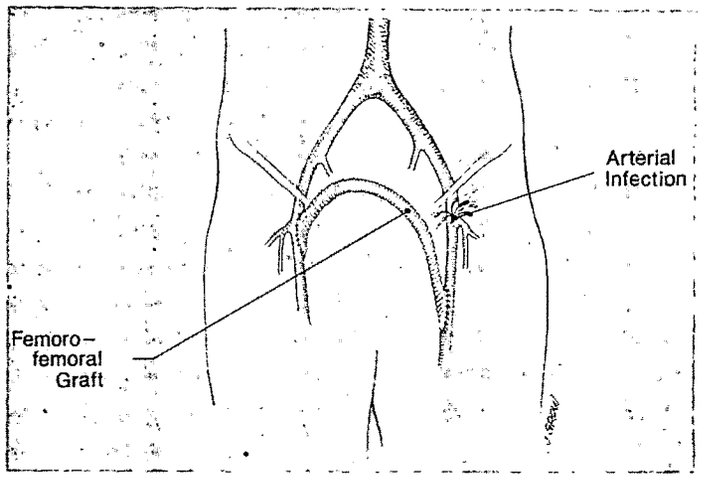


Fig. 2. The cross-over femoro-femoral graft as illustrated here allows maintenance of arterial continuity during direct treatment of these surgical infections.

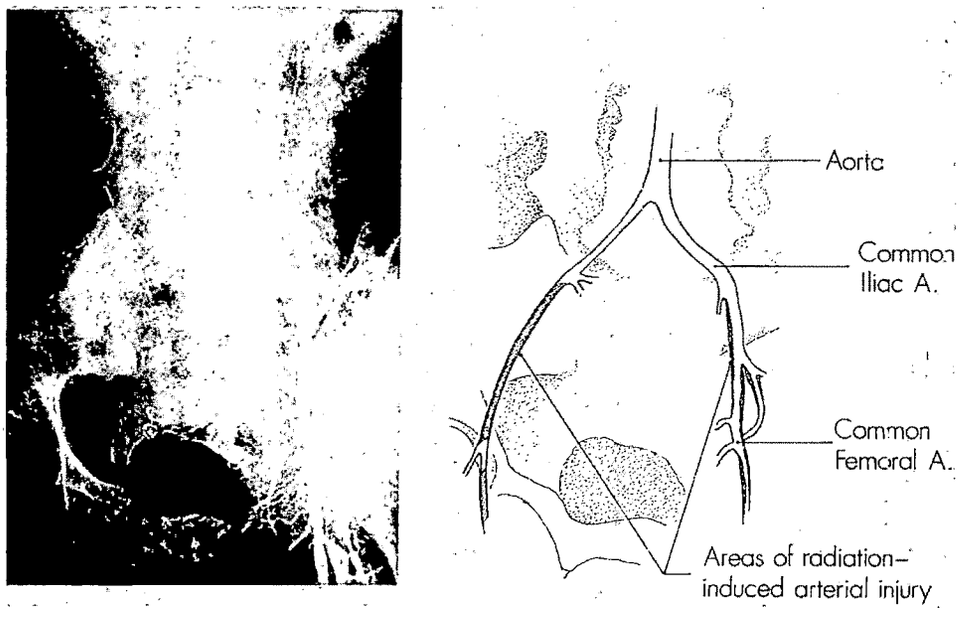


Fig. 3. This arteriogram shows radiation-induced injury to the external iliac arteries, producing occlusion on the right and stenosis on the left.

the lesion. If the lesion proves to be intimal fracture or arterial thrombosis, direct repair is undertaken. If the lesion is an infectious erosion with intrapelvic hemorrhage, a cross-over graft is performed through a clean field before the infected artery is ligated proximally and distally.

Extra-anatomic arterial repair

Infectious erosion of the common femoral artery following groin lymphadenectomy with and without radical vulvectomy has decreased in frequency. It has become generally appreciated that vascular coverage is of paramount importance and, in particular, the technique of sartorius muscle mobilization to effect arterial coverage has been of value.[3] Nevertheless, the combination of irradiation, delayed surgical wound healing, and infection has caused—and will continue to cause—a number of femoral arterial injuries. Vascular surgeons have become experienced at performing extra-anatomical bypass procedures for repair of arterial injury and occlusion.[4] The situation of femoral artery infection is unfortunately familar to experienced vascular surgeons. It is treated by maintenance of arterial continuity while excluding the infection from the arterial stream. In principle, the direct arterial ablation is usually preceded by sterile perfor-

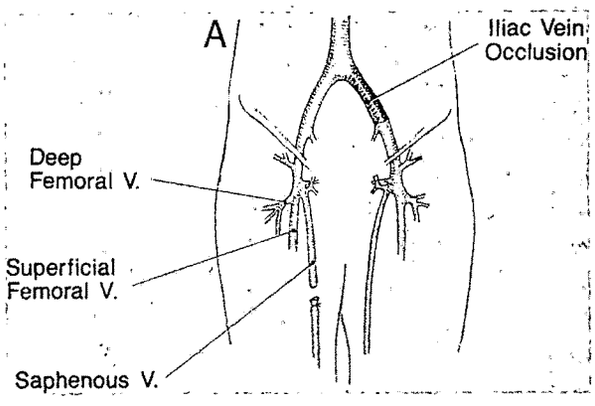


Fig. 4A. Venous reconstruction is performed for chronic limb swelling secondary to major venous occlusion from surgical treatment with or without radiation therapy. As seen here, the saphenous vein is detached distally in the contralateral thigh.

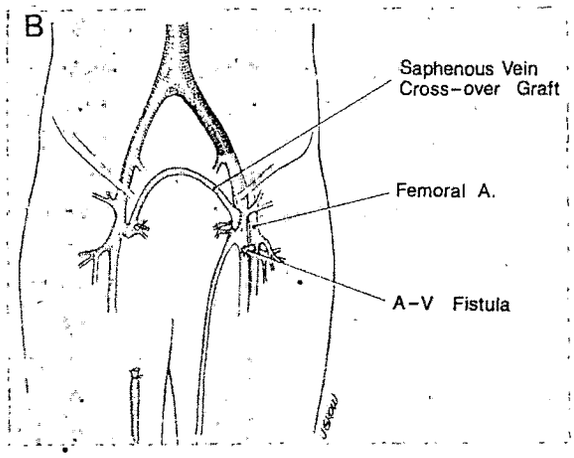


Fig. 4B. The contralateral saphenous vein is then crossed over subcutaneously to be anastomosed to the common femoral vein on the affected side. Creation of a temporary arteriovenous fistula insures patency of the reconstruction.

mance of a cross-over graft. The donor artery may be the contralateral external iliac artery in the pelvis or the common femoral artery in the groin, depending upon the patient's own surgical situation (Fig. 2). A poorly healed contralateral groin incision would mitigate against the use of this vessel as a donor artery and the surgeon would have to enter the pelvis through an anterior incision much like a modified inguinal hernia incision. Following this, the graft is usually led subcutaneously suprapublically across the groin crease medial to the femoral triangle, and then anastomosed to the superficial femoral artery in the midthigh, well distal to the affected area. Following this type of arterial reconstruction the common femoral artery can be attacked directly and the infection evacuated and exteriorized while the artery itself is ligated cleanly above and below the infection.

Extra-anatomic bypass techniques such as the one described have a remarkable record of long-term success, and can be applied judiciously before actual hemorrhage occurs from the infected artery.

Radiation arterial injury

It is not commonly appreciated that radiation injury can produce delayed arterial occlusion. Fig. 3 shows the arteriogram of such a patient, with external iliac artery occlusion on the right and marked irregularity of the external iliac artery on the left. These anatomic segments of arterial involvement are rarely affected by atherosclerotic occlusive disease but are precisely in the area of irradiation in this particular patient.

This patient received a total midpelvic tumor dose of 6,000 rads distributed through four 12 by 15 opposing pelvic ports at a 45 degree angulation. She sustained progressive arterial occlusion, documented by the ultrasound technique. The occlusion worsened rapidly in the 6 months prior to need for repair, and finally eventuated in total right external iliac artery blockage. Repair was by conventional aortofemoral bypass grafting.

Such arterial injury has been described.[5] Histopathologically, the arterial changes consist of distortion and destruction of the elastic lamina, collagen injury secondary to the inflammation of radiation, and late degeneration and calcification of the media. In the experimental animal, it has been possible to produce these changes with doses ranging from 1,000 to 9,500 rads.[6]

Venous injury

There are two aspects of venous injury which concern the pelvic surgeon. One is venous thrombosis, similar to that which may occur in any surgical patient; the other is chronic venous insufficiency produced by surgical, radiation, or infectious venous injury.

Prophylaxis of venous thrombosis has been investigated quite accurately in recent years. The advent of nuclide tagging of human fibrinogen has allowed administration of such material prior to the surgical event in large patient populations. Thus, a number of prophylactic measures have been tested. Among these are active and passive exercise, wearing of knee-length or thigh-length elastic stockings, administration of prothrombin-depressant anticoagulation, or heparin. Heparin has been tested both in full therapeutic doses which affect the Lee-White clotting time and in subclinical or "miniheparin" dosage, which does not affect laboratory determination of clotting time.

Of all these methods, the one that has been proved to show reduction in venous thrombosis without great

risk of hemorrhage has been the "miniheparin" schedule proposed by Kakkar and associates[7] of King's College Hospital in London. This schedule advises administration of 5,000 units of heparin subcutaneously immediately before surgery, followed by ten doses at 12 hour intervals. Such heparin dosage decreases (but does not abolish) the rate of formation of calf venous thrombosis. Similarly, it decreases (but does not eradicate) the incidence of postoperative thromboembolic disease.

Thus, prophylaxis of venous thrombosis has been improved as methods of detection of venous clot have also improved. Tagged fibrinogen has the unfortunate liability that it must be given to a patient before clots form. Therefore, there has been interest in developing techniques for diagnosis of venous thrombosis by noninvasive or minimally invasive methods. Of these, the one which shows the greatest promise is administration of technetium-tagged human albumin microspheres through dorsal foot veins. This allows isotope venography and simultaneous lung scanning. [8]

This method has a high degree of correlation with contrast venography and also has the additional advantage that the human albumin microspheres seem to have an affinity for partial venous occlusions caused by thrombi. These are visualized as "hot spots" on the isotope venogram. When such a technique is combined with lung scanning, it has been possible to determine the presence of venous thrombosis in patients who were otherwise asymptomatic.[9] Similarly, unsuspected pulmonary emboli have been visualized in patients whose only complaint was limb swelling.

Venous reconstruction

Reconstructive surgery of large veins has lagged far behind success in repairing arteries. Just as more precision has been achieved in diagnosis of venous occlusion, so too has reconstruction of venous segments begun to be a part of the vascular surgeon's repertoire. At present, the patient with a massively swollen limb who is not responsive to conservative measures of firm elastic support is investigated by venography, ultrasound, and impedance plethysmography. If a pelvic venous occlusion is seen, plans are made for direct venous bypass or cross-leg venous bypass. These techniques have been advocated in the past but have not met with wide acceptance, largely because surgery of the venous system has been fraught with thrombosis. Although surgeons advocating these procedures have pointed out quite correctly that such thrombosis does not preclude recanalization and recovery of patency, there has not been a great wave of enthusiasm for the performance of such operations.

As experience has increased on the Continent, with the use of distal arteriovenous fistula concomitant with direct venous reconstruction, the success rate as determined by venous patency has improved (Figs. 4*A* and 4*B*). Typically, a patient with a left iliac venous occlusion now has the right saphenous vein mobilized from knee to groin and then passed as a suprapubic cross-over graft to the common femoral vein on the affected side. With a single venous anastomosis being performed between the distal saphenous vein from the right and the common femoral vein on the left, blood is rerouted around the iliac venous occlusion and up into the right iliac venous segment (Figs 4*A* and 4*B*).

Such a reconstruction would be expected to thrombose were it not for the fact that a concomitant arteriovenous fistula is created between the superficial femoral artery on the left and the nearby femoral vein. This fistula is created with the use of a 5 mm. tubular saphenous vein autograft and the rate of flow through such a fistula insures that thrombosis of the cross-over graft will not occur. After 6 to 8 weeks, the fistula is divided and the patient is allowed to wear minimum-support stockings.

The results with such procedures have been dramatically successful and patients with massive venous edema have been rehabilitated to near normal living.

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Plasma levels of norepinephrine (NE) during the periovulatory period and after LH-RH stimulation in women

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In three normally cycling women studied daily from day 10 to day 17 of the menstrual cycle, the levels of circulating norepinephrine showed a sharp rise preceding or concomitantly with the ovulatory LH surge. In two patients the norepinephrine peak took place 24 hr. previously to the LH rise and in the third one it occurred simultaneously. The simultaneous determination of ovarian hormones and norepinephrine showed no temporal correlation between this catecholamine and either estradiol or progesterone. On the other hand, after a single intravenous 100 μg dose of LH-RH, a significant rise in plasma norepinephrine, preceding the LH peak, was found in the four patients studied. The determination of norepinephrine at 3 minute intervals beginning one minute after LH-RH injection showed a significant rise in the amine levels ranging from 5 to 10 times in respect to basal values between 1 and 6 minutes after LH-RH stimulation. In these patients a second peak of norepinephrine occurred simultaneously with the maximal response of LH, which rose to peak levels after 18 minutes in one patient and after 24 minutes in the other. These findings are discussed with respect to the origin and role of increased amounts of plasma norepinephrine related to the LH surge.

THE OCCURRENCE of ovulation, the central event in the reproductive physiology of the female, remains until now uncertain. The main approach to the problem has been focused either on the determination of hypophysial and ovarian hormones or by evaluating the physiologic modifications caused by these hormones.

Even though one of the most important responsibilities of the autonomic nervous system is the control of gonadotropin secretion,[1, 2] which leads to both follicular growth and ovulation, the role of this system and particularly norepinephrine in the reproductive physiology of women has been until now unknown. In the rat, during the estrous cycle, castration, and pregnancy, the changes in the concentration of gonadotropins and ovarian hormones in blood are correlated with modifications in the hypothalamic catecholamine content and MAO activity.[3, 5] On the other hand, it has been observed that drugs which deplete the brain of catecholamines or inhibit their synthesis could block ovulation, [6, 8] indicating that catecholamines participate in this process. Presumably,

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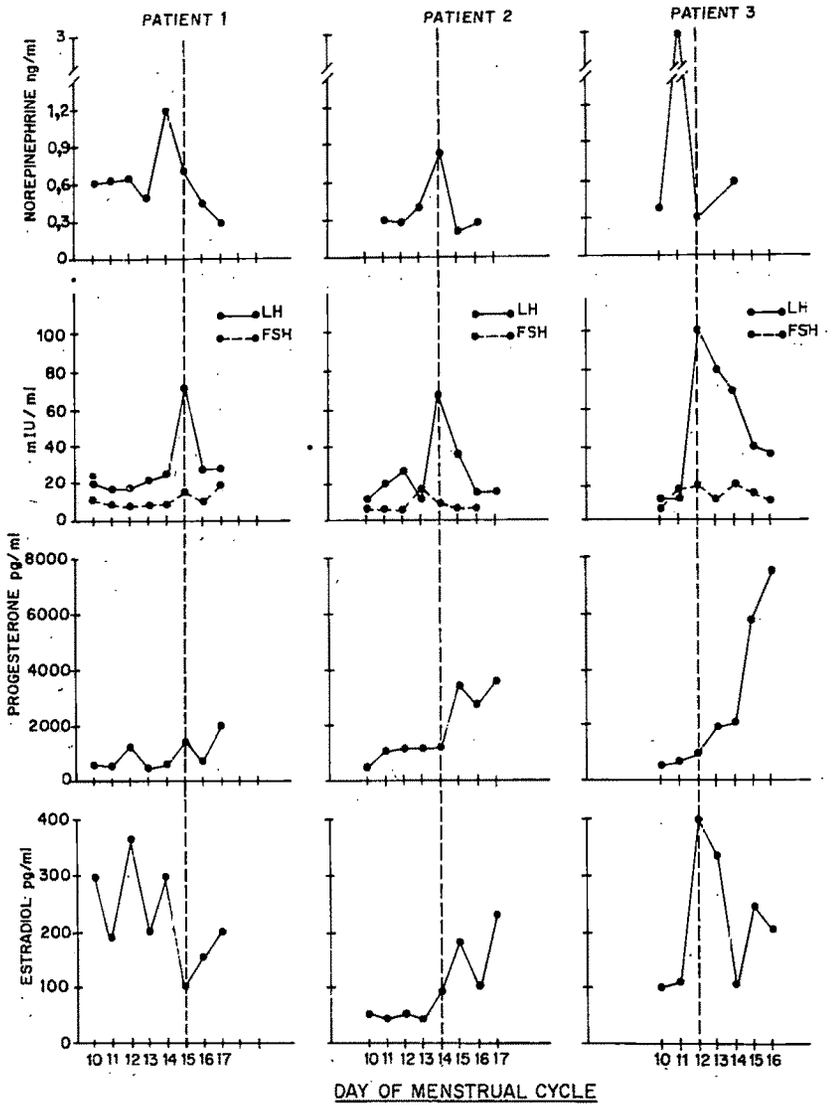


Fig. 1. Patterns of plasma norepinephrine, gonadotropins, progesterone, and estradiol in three normal cycling women studied from days 10 to 17 of the menstrual cycle, with apparent ovulation. Plasma levels of progesterone on day 24 were: 13,600 pg./ml. for Patient 1, 12,000 pg./ml. for Patient 2, and 15,000 pg./ml. for Patient 3.

more than one transmitter is involved in the secretion of gonadotropins. Norepinephrine, however, appears to be the synaptic transmitter mediating the release of LH.[9]

Recently, Zuspan and Zuspan,[10] reported elevated plasma norepinephrine levels associated with the time of ovulation in women. Since norepinephrine is primarily a reflection of the sympathetic nervous sytem activity, this finding could be related with the release of amines from the nerve stores that ultimately result in the release of LH. The development of a highly sensitive radioenzymatic assay for norepinephrine in plasma[11] has afforded an opportunity to investigate the following: (1) daily plasma levels of norepinephrine and correlation of them with the plasma levels of FSH, LH, estradiol, and progesterone, during the periovulatory period, and (2) effects of a single intravenous rapid injection of LH-RH on the same parameters.

Material and methods

Periovulatory period studies. Three normally cycling women were studied from days 10 to 17 and on day 24 of the menstrual cycle. For collection of blood samples, the patients lay in a supine position for at least 20 min. A tourniquet was placed around the arm, and

blood was drawn from the antecubital vein without the patient exerting muscular activity of the forearm. Blood samples were collected daily at 9 A.M. with heparin and placed in a tube containing solid reduced glutathione (final concentration, 5 mM.). The plasma was quickly separated by centrifugation, decanted, and frozen.

LH-RH stimulation studies. For the LH-RH studies four normally cycling women maintained in the conditions described above received, on day 10 of the menstrual cycle, a single intravenous injection of 100 μg of synthetic LH-RH (Hoescht) dissolved in 1 ml. of saline solution. In two patients, blood samples were withdrawn before the administration of LH-RH and at 15, 30, 45, and 60 min. after the injection. In the other two patients blood was drawn in the basal state and at 3 min. intervals for 30 min., followed by an additional sample 45 min. after the LH-RH injection.

Plasma samples for NE determinations were recentrifuged at 4° C. for 10 min. at 30,000 x g and the supernatant was assayed directly. Norepinephrine concentration was measured by a highly sensitive radiometric assay, based on the enzymatic conversion of norepinephrine to its O-methylated analogues in the presence of catechol-O-methyltransferase and S-adenosyl methionine-methyl-^{3}H. Following purification of the labeled normetanephrine by solvent extraction and thin-layer chromatography; the amine is oxidized to vanillin, purified by solvent extraction, and counted.[11] Results are expressed in ng./ml. of plasma.

Plasma FSH and LH levels were determined by triplicate by a double antibody radioimmunoassay technique (Serono Immunochemicals, Italy) and expressed as milli-International Units of Second International Reference Preparation Human menopausal gonadotropin second IRP-HMG) per ml. of plasma (m.I.U./ml.).

Plasma progesterone was measured in triplicate by a double-antibody radioimmunoassay, without chromatography,[12] using a highly specific antibody. Results are expressed in pg./ml. of plasma. Plasma estradiol levels were measured by a double-antibody radioimmunoassay method.[13] Estradiol was isolated by thin-layer chromatography in benzene-ethyl acetate (90:40, v/v) system. Results are expressed in pg./ml. of plasma.

Results

Periovulatory period. The individual patterns of circulating NE, FSH, LH, progesterone, and estradiol levels in three normally cycling women from day 10 to day 17 are shown in Fig. 1. The day of the LH peak was used as a reference point for plotting all the data.

Plasma norepinephrine had a mean level of about

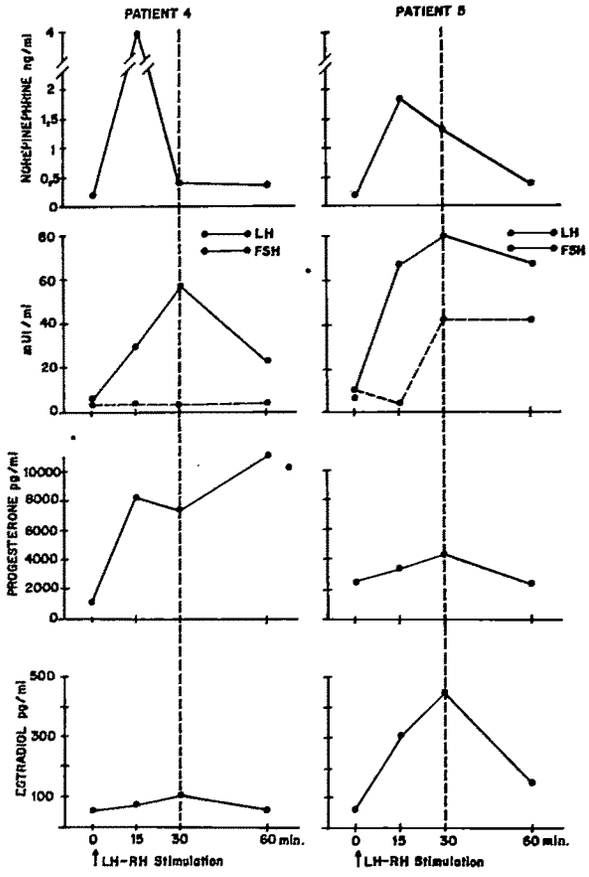


Fig. 2. Patterns of plasma norepinephrine, gonadotropins, progesterone, and estradiol in two normal cycling women previously and after a single intravenous injection of 100 μg of synthetic LH-RH on day 10 of the menstrual cycle.

0.3 to 0.6 mg./ml. prior and after the "critical period." One day prior to or concomitant with LH peak, plasma norepinephrine began to rise rapidly to reach its maximum values. The plasma peak of norepinephrine occurred one day prior to LH peak in two subjects and on the day of the LH peak in one subject. The peak norepinephrine levels ranged from 0.7 to 3.0 ng./ml. Plasma FSH levels remained practically unaffected in the three patients studied.

Plasma levels of progesterone were about 500 pg./ml. in the late follicular phase. Plasma progesterone began to rise either on the day of LH peak or 24 hr. later to reach levels of 2,000 to 2,500 pg./ml. two days after the LH surge, with a subsequent rapid rise to values of 3,500 pg./ml. on day 3 and 7,000 pg./ml. on day 4 post-LH peak. On day 24 progesterone levels ranged between 12,000 and 15,000 pg./ml. of plasma.

The pattern of plasma estradiol showed individual variations. In two subjects plasma estradiol had a mean level of about 50 to 100 pg./ml. during the late follicular phase. Plasma estradiol began to rise rapidly

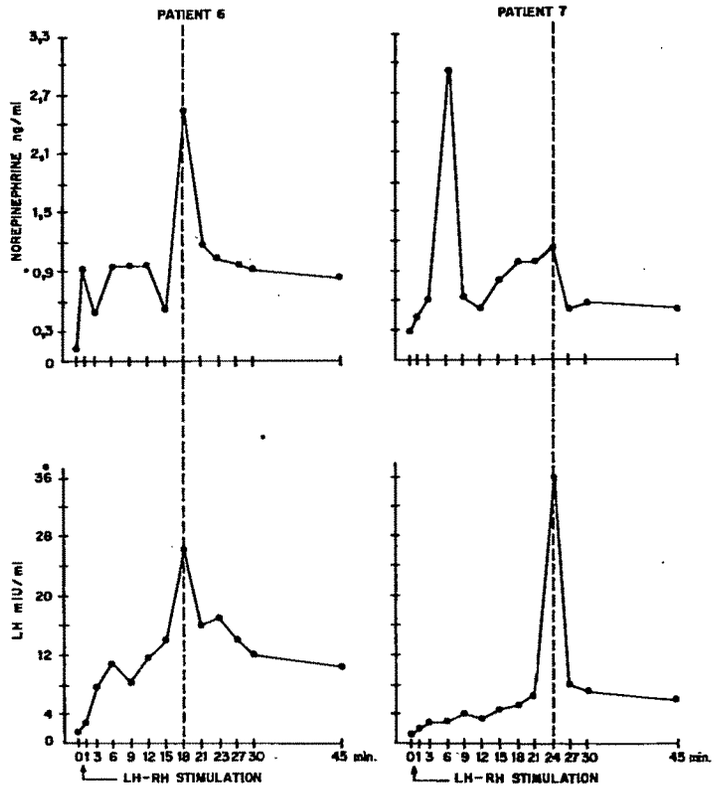


Fig. 3. Patterns of plasma norepinephrine and LH in two normal cycling women previously and after a single intravenous injection of 100 μg of synthetic LH-RH on day 10 of the menstrual cycle.

to reach a peak level on the day of LH peak in one patient and 24 hr. after, in the other. The peak estradiol levels ranged from 200 to 400 pg./ml. falling rapidly to reach a level of 100 pg. two days post-LH peak. From day 2 post-LH peak estradiol began to rise to reach levels between 200 and 250 pg./ml. on days 3 and 4 after LH surge. In the third subject, plasma-estradiol had a level between 200 and 350 pg./ml. in the late follicular phase. On the day of LH-peak, estradiol fell rapidly to reach a level of 100 pg. with a subsequent rise to reach a level of 200 pg. 2 days after LH surge.

Stimulation with LH-RH. Fig. 2 shows the studies in two normal cycling women, in which NE, FSH, LH, estradiol, and progesterone levels were measured at different times after the LHRH injection. Plasma norepinephrine had a mean level of about 0.1 to 0.2 ng./ml. in the basal state. After LH-RH injection the norepinephrine level rose rapidly to 4 ng./ml. in one subject and 1.7 ng./ml. in the other. The maximum increase in norepinephrine occurred 15 min. after LH-RH. Following the peak, plasma norepinephrine levels began to fall rapidly to reach a mean level of about 0.35 ng./ml., 60 min. after the LH-RH injection. As previously reported, an increase in LH levels was observed. In both patients the maximum increase in plasma LH occurred 30 min. after the stimulation.

Plasma FSH levels remained unaffected (low) in one of the subjects whereas in the other, FSH increased to reach peak levels, 30 min. after the LH-RH injection.

Plasma progesterone basal levels were 1,000 and 2,500 pg./ml., respectively. After LH-RH injection the progesterone levels rose to peak level at 60 min. in one subject and at 30 min. in the other. Estradiol levels followed the same profile as LH in both subjects.

The results of the study in which LH-RH was administered to two normal cycling women and plasma levels of LH and norepinephrine determined at 3 min. intervals, beginning one minute after LH-RH injection, are shown in Fig. 3.

Plasma norepinephrine had a basal level of about 0.1 to 0.3 ng./ml. In one patient plasma norepinephrine began to rise rapidly to reach a level of about 1 ng./ml. one minute after LH-RH injection. Following a fall in the levels of norepinephrine at the 3 min. point, a subsequent rise occurred at 6 min. with a plateau that

persisted from 6 until 12 min. post LH-RH injection. After a fall in the norepinephrine levels at 15 min., plasma norepinephrine rose to reach a peak level of about 3 ng. on the eighteenth minute post LH-RH injection. After the peak, norepinephrine began to fall to reach levels of 0.9 ng. 45 min. after LH-RH injection. In the other patient plasma norepinephrine began to rise 1 min. after LH-RH injection to reach a peak of about 3 ng./ml. on the sixth min. post LH-RH injection. Following the initial peak of plasma norepinephrine levels, a sharp drop occurred to reach a level at 9 min. post LH-RH of about 0.55 ng./ml. with a subsequent rise to reach a second peak of about 1 ng./ml. at 24 min. Norepinephrine levels fell to reach levels of about 0.5 ng./ml. between 27 and 45 min. Plasma LH levels began to rise 1 min. after LH-RH injection. The maximum increase in plasma LH occurred after 18 min. in one patient and after 24 min. in the other.

Comment

The present data demonstrate that in the periovulatory period the levels of circulating norepinephrine showed a sharp rise varying from 2 to 7 times with relation to basal values. In two of the three studied patients the NE peak took place 24 hr. previously to the LH rise and in the third one it occurred simultaneously. The assumption that ovulation had occurred was based on the presence of a mid-cycle peak of LH and by elevated progesterone plasma levels in the twenty-fourth day of the menstrual cycle. This finding is in agreement with the observation of Zuspan and Zuspan,[10] who described a plasma norepinephrine surge that precedes or is associated with increased levels of LH. The simultaneous determination of ovarian hormones and norepinephrine showed no temporal correlation between this catecholamine and either estradiol or progesterone, even though a possible relationship between them cannot be discarded because a 24 hr. sampling can overcome the steroids variation that takes place more frequently.

Since synthetic LH-RH stimulates the release of LH into the circulation[14] and the amount of liberated LH is comparable to the normally occurring levels during the LH-surge, experiments were designed in order to determine if the release of LH in response to a single acute LH-RH injection is associated with a rise in plasma norepinephrine concentration. The employment of a highly sensitive and specific radioenzymatic technique has allowed us to make frequent samplings and to determine NE in small volumes of plasma. After a single 100 μg dose of LH-RH, a significant rise in NE was found in both normal women. The maximal response was obtained at 15 min. after LH-RH whereas the maximal increase in LH was observed at 30 min. The rise in NE was abrupt and sharp, falling back to basal values at 60 min. The possibility that the rise of NE could occur before the first determination at 15 min. was analyzed by stimulating two other normal cycling women in an identical manner but collecting blood 1 min. after LH-RH injection and every 3 min. for 30 min. A significant rise in NE, preceding the LH peak was found in both patients at 1 and 6 min. respectively after the LH-RH injection. In both patients a second peak of NE that occurred simultaneously with the LH peak was observed at 18 and 24 min. respectively.

At present a cause-to-effect relationship between norepinephrine and the LH surge cannot be ascertained. The large body of experimental evidence implicating the norepinephrine in the regulation of LH secretion by the anterior pituitary,[15] however, suggest several questions about the origin and role of the increased amount of plasma norepinephrine related to the LH surge. For example, as previously suggested by Zuspan and Zuspan,[10] it is possible that the increased plasma amine levels during the periovulatory period reflect an activation of the norepinephrine-contained terminals located in the hypothalamus which brings about the discharge of LH-RH into the portal vessels and consequently the LH secretion by the anterior hypophysis. However, our findings in LH-RH stimulated patients seem to indicate that the plasma norepinephrine rise occurs as a result of the LH-RH action.

A possible effect of synthetic LH-RH on the activity of noradrenergic neurons which could determine an increase of the amine in plasma cannot be ruled out since another releasing factor, TRH, enhances the turnover of norepinephrine in the brain.[16]

On the other hand, a possible peripheric role of the rise of the plasmatic amine should also be considered. It could be speculated that the increment in plasma norepinephrine concentration that occurs associated with the periovulatory LH surge could modify the uptake of this amine by the reproductive tract causing modifications that ultimately result in functional changes of these organs in relation to ovulation.

In any event, further work will be necessary to determine if the rise in plasma norepinephrine that occurs in the periovulatory period or after LH-RH stimulation reflects a central phenomenon from which results the LH secretion and consequently the ovulation.

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Contraceptive choice and prevalence of cervical dysplasia and carcinoma in situ

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Whether use of oral contraceptives is associated with subsequent development of cervical neoplasia is an important public health question. Before evaluating this issue, we must determine if choosing oral contraceptives identifies a woman who is intrinsically at high risk of developing cervical neoplasia. We have examined the demographic and reproductive characteristics as well as cervical premalignant changes manifest by 15- to 44-year-old black women who enrolled in a public family-planning clinic. When compared to IUD acceptors, oral contraceptive acceptors did not have a disproportionately large number of women with traits which predicted for high risk of developing cervical neoplasia. Oral contraceptive acceptors, compared to IUD acceptors, did not have a higher prevalence rate of carcinoma in situ. Oral contraceptive acceptors with no Pap smears prior to initial choice of contraceptive method did have a 1.4-fold higher prevalence rate of cervical dysplasia than IUD acceptors. These results suggest that the decision to use oral contraceptives, per se, does not identify a group of women at higher risk to develop cervical carcinoma in situ. Such a decision may, however, identify a group with a small increased propensity for developing cervical dysplasia.

RECENT STUDIES have examined whether continued use of oral contraceptives is associated with development of cervical neoplasia.[1–4] However, before this can be resolved, the problem of self-selection of contraceptive methods must be examined. We must determine whether by choosing to use oral contraceptives a women identifies herself as being at higher risk of developing cervical premalignant changes (dysplasia and carcinoma in situ) than a woman who chooses an intrauterine device (IUD). Three recent studies have examined this problem, but came to different conclusions.[5–7]

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The present report examines three questions: (1) What demographic traits are risk indicators for cervical premalignant changes? (2) Do initial acceptance rates of oral contraceptives and IUDs vary disproportionately with respect to these risk indicators? (3) Does the prevalence rate (manifest risk) of cervical premalignant changes vary based on which contraceptive method is initially accepted by women entering a family-planning program?

Materials and methods

The subjects are drawn from women who enrolled in the Grady Memorial Hospital Family-Planning Clinic in Atlanta, Georgia, between 1967 and 1972. To receive a Grady Hospital number, each woman must be certified as eligible to receive services from Grady Hospital. Grady Hospital's eligibility criteria assure that patients are of low socioeconomic status. Further proof that subjects are of low socioeconomic status comes from a 1971 citywide family planning survey. Of 247 women who had a Grady Hospital number, 266 (91 per cent) were from the lower or lower-middle class census tracts.[8]

Table I. Age specific cervical dysplasia and carcinoma in situ prevalence rates* in IUD and oral contraceptive acceptors

Age	Total women	Biopsy confirmed	
		Dysplasia	Carcinoma in situ
15-19	9,034	1.7	0.1
20-24	6,820	2.5	0.3
25-29	3,371	2.5	0.6
30+	2,915	2.4	1.0
Total	22,140	2.1	0.4

*All rates are per 100 women.

Table II. Prevalence rates* of cervical dysplasia by patient characteristics

Marital status						
	Currently married	*Never married*	*Formerly married*			
	2.5	2.5	3.4			
Age at first pregnancy						
	< 18	*≥ 18*	*Unknown*			
	2.9	1.2	3.0			
Yrs. of school completed						
	1-8	*9-10*	*11-12*	*13+*	*Unknown*	
	3.7	2.9	2.7	2.5	2.2	
Yr. of entry to family planning						
	1967	*1968*	*1969*	*1970*	*1971*	*1972*
	1.0	1.7	3.5	3.6	3.5	3.1
No. of pregnancies						
	0	*1*	*2*	*3*	*4+*	*Unknown*
	2.9	1.4	3.6	3.3	3.2	2.4
No. of Pap smears prior to entering family planning						
	0	*1+*				
	3.1	2.3				

*All rates are age-standardized and given per 100 women.

At her first clinic visit, each woman completes a questionnaire about age, marital status, education, and reproductive experience. A medical history is taken, a physical examination is administered, and a Pap smear of cervical cells is taken. Each woman selects a contraceptive and, unless contraindicated, that method is prescribed.

In 1967 and part of 1968 no reproductive or education information was obtained. It remains unavailable on approximately 22 per cent of women. We searched Grady Hospital records for the pregnancy history of a systematic 1-in-20 sample of subjects with unknown number of pregnancies. They were older (27.0 ± 6.2) and had more pregnancies (4.1 ± 2.7) than those whose number of pregnancies was known (22.7 ± 6.1 and 2.4 ± 2.1, average age and parity, respectively). These 22 per cent were retained in all analyses. When looking for effects of education or parity, we put the "unknowns" in a separate stratum.

We recorded the reported results of all cervical Pap smears and biopsies done at Grady Hospital since 1965, and all family-planning records since 1967. Because both master files identify women by Grady Hospital number, it was possible to link records from both files.

To test the accuracy of the linkage of records from two separate files, we compared the names on matching records. Mismatches occurred on 1.1 per cent of the linkages. In addition, we examined over 1,300 records from our cytopathology master file to see how accurately it reflects the cytology and pathology log books. There was less than 2 per cent disparity between the logs and our master file, and we were equally likely to over- or under-record the actual reading. The mismatches and transcription errors occurred proportionately among acceptors of different contraceptive methods. The family-planning record system is machine edited for inconsistent data. Since most women make more than one visit, missing or inaccurate data can often be corrected from subsequent visit records.

Of the 31,981 women enrolled in the Family-Planning Clinic from 1967 to 1972, we wished to study black women between 15 and 44 years of age with at least one adequate Pap smear. We identified 25,232 such women. Of these, 22,140 (87.7 per cent) accepted either the IUD (6,663) or oral contraceptives (15,447); 5.5 per cent accepted injections of dehydromedroxyprogesterone acetate and 6.8 per cent used a barrier contraceptive—foam, condom, or diaphragm.

Subjects were classified by cytopathologic history up to and including the family-planning admission Pap smear. The categories were "all entries normal"; or biopsy proved atypia, dysplasia, carcinoma in situ, or invasive cancer of the cervix. There were also 81 women with abnormal Pap smears and either no follow-up or normal follow-up. These women were distributed proportionately by all demographic variables considered. Further checking of these 81 revealed that more than 70 per cent had subsequent normal Pap smears. We presumed this group to be "false positives" and have retained them in the analysis as normals. Finally, if a woman's first abnormal Pap smear occurred on admission to family planning, her records were reviewed for six more months to see if the abnormality was subsequently biopsy confirmed.

Unless otherwise specified all rates are age standardized by the direct method. The age distribution of all 25,252 women is used as the standard. We employ the

standardized risk ratio to compare standarized rates.[9] To test the significance of the deviation of the observed risk ratio from the expected 1.0, we compute 95 per cent confidence limits.[10] If these limits do not include 1.0, the observed risk ratio is significantly ($p < 0.05$, 2-tail) different from 1.0.

Results

Table I shows that dysplasia prevalence increased from 1.7 per 100 in women 15 to 19 years old to 2.5 per 100 in women in their twenties and older. The carcinoma in situ prevalence rates rise steadily from 0.1 per 100 in the youngest women to 1.0 per 100 in the oldest subjects.

Summarized in Table II are the age-standardized prevalence rates of cervical dysplasia by patient characteristics. Highest rates (per 100) of dysplasia are found in those women who: were formerly married (3.4); became pregnant before age 18 (2.9); completed less than 9 years of school (3.7); enrolled in family planning after 1968 (approximately 3.4); had two or more pregnancies (approximately 3.3); and had had no Pap smears prior to enrolling in family planning (3.1).

Table III is similar to Table II, except it presents age standardized prevalence rates for cervical carcinoma in situ. The highest rates of carcinoma in situ are found in those women who: were formerly married (0.8); became pregnant prior to age 18 (3.0); completed less than 9 years of school (0.9); have 3 or more pregnancies (0.9); and had no Pap smears prior to enrolling in family planning (0.9).

The acceptance of the IUD increased from 24 per cent of women in the youngest age group to 34 per cent in the oldest group (Table IV). Acceptance of oral contraceptives decreased from 69 per cent in the youngest to 42 per cent in the oldest age group. Choice of contraceptive method did not vary substantially with respect to marital status or age at first pregnancy. Of women with less than an eighth grade education, 25 per cent chose the IUD. In contrast, of women who completed high school (or more), 19 per cent chose the IUD. The comparable rates for oral contraceptive acceptors are 59 and 68 per cent. Of women entering the program in 1967, 40 per cent chose the IUD. By 1972 only 15 per cent of entering women accepted that method. Comparable acceptance rates for oral contraceptives are 55 and 67 per cent. Ten per cent of nulliparous women accepted the IUD, while 28 per cent of women with four or more pregnancies accepted that method. The comparable acceptance rates for oral contraceptives are 81 and 51 per cent. For women who enter the clinic having had no previous Pap smears, 17 per cent chose the IUD. In those enrollees with previous Pap smears, 31 per cent chose the IUD. Comparable rates for those choosing oral contraceptives are 68 and 58 per cent.

In short, the older, less well-educated, highly parous women who enrolled in the clinic in the late 1960's having had previous Pap smears were more apt to choose the IUD than oral contraceptives.

Table V shows the prevalence of dysplasia in acceptors stratified by number of Pap smears. For women with one Pap smear, the crude dysplasia prevalence rate was 2.0 per 100 IUD choosers. Among similar oral choosers, the dysplasia prevalence rate was 2.7. The standardized ratio of these dysplasia rates (oral contraceptive to IUD acceptors) was 1.4, and 95 per cent confidence limits lying between 0.9 and 2.1. In women with two or more Pap smears, choosers of oral

Table III. Prevalence rates* of cervical carcinoma in situ by patient characteristics

Marital status	*Currently married*	*Never married*	*Formerly married*			
	0.6	0.7	0.8			
Age at first pregnancy	*< 18*	*≥ 18*	*Unknown*			
	3.0	0.4	0.6			
Yrs. of school completed	*1-8*	*9-10*	*11-12*	*≥13*	*Unknown*	
	0.9	0.8	0.6	0.1	0.4	
Yr. of entry to family planning	*1967*	*1968*	*1969*	*1970*	*1971*	*1972*
	0.4	0.5	0.6	0.5	0.7	1.0
No. of pregnancies	*0*	*1*	*2*	*3*	*≥4*	*Unknown*
	0.4	0.6	0.5	0.9	0.9	0.4
No. of Pap smears prior to entering family planning	*0*	*≥ 1*				
	0.9	0.4				

*All rates age standardized and per 100 women.

Table IV. Age specific acceptance rates by contraceptive chosen*

		Contraceptive chosen	
Age	*Total women*	*IUD*	*Oral*
15-19	9,706	24.2	68.9
20-24	7,682	23.9	64.9
25-29	3,972	29.5	55.4
30+	3,872	33.8	41.5
Total	25,232	26.4	61.3

*This table includes all eligible women and shows acceptance rates per 100 women.

Table V. Prevalence rates* of cervical dysplasia by initial contraception method chosen stratified by number of Pap smears

No. of Pap smears	*Method chosen*	*Total women*	*Crude rate**	*Risk ratio†*	*95 per cent confidence limits*
1	IUD	1,499	2.0		
	Oral	6,046	2.7	1.4	(0.9-2.1)
2+	IUD	5,164	1.3		
	Oral	9,431	2.3	1.5	(1.1-1.9)
Total	IUD	6,663	1.5		
	Oral	15,477	2.4		

*All rates are per 100 women.

†Standardized for age at entry and year of entry.

Table VI. Prevalence rates* of carcinoma in situ of the cervix by initial contraceptive method chosen stratified by number of Pap smears

No. of Pap smears	*Method chosen*	*Total women*	*Crude rate**	*Risk ratio†*	*95 per cent confidence limits*
1	IUD	1,499	0.92		
	Oral	6,046	0.34	0.6	(0.3-1.2)
2+	IUD	5,164	0.31		(0.7-2.1)
	Oral	9,431	0.34	1.2	
Total	IUD	6,663	0.45		
	Oral	15,477	0.34		

*All rates are per 100 women.

†Standardization for age at entry and year of entry.

contraceptives had a significantly higher prevalence of dysplasia than choosers of IUD's by a factor of 1.5 (95 per cent confidence limits: 1.1 to 1.9). To prevent confounding, we had to standardize the comparison of rates for age and year of entry to family planning. Further standardization for the effects of the other variables on which we had information did not substantially alter the standardized risk ratios that are presented in Table V.

Table VI is comparable to Table V, except that it shows the prevalence of carcinoma in situ. For subjects with one Pap smear, oral choosers had 0.6 (0.3 to 1.2) the prevalence of carcinoma in situ when compared to IUD choosers. (The comparison was standardized for age and year of entry for reasons already discussed.) For women with two or more Pap smears, the comparable ratio of standardized rates was 1.2 (0.7 to 2.1).

Comment

The patient characteristics that were associated with high prevalence rates (risk indicators) of dysplasia were even more strongly associated with carcinoma in situ. For example, the dysplasia prevalence in the most highly educated was 50 per cent lower than in the poorest educated. A similar comparison of carcinoma in situ rates shows a nine-fold lowering between the most and the least educated. The associations of cervical carcinoma in situ with older age, higher parity, low education, and various states of marital dissolution are consistent with current knowledge.[11] In addition knowing that dysplasia has epidemiologic characteristics similar to carcinoma in situ is consistent with the argument that dysplasia is a precursor of carcinoma in situ.

Some of these risk indicators for cervical disease also predicted for IUD acceptance—low educational attainment, high parity, and older age. Other risk indicators—no previous Pap smears and enrolling in the Grady Hospital Family-Planning Clinic in the 1970's—predicted for oral contraceptive acceptance. Clearly, the group of women who choose orals does not have a disproportionately large number of women with attributes predicting high risk of developing cervical neoplasia when compared to IUD choosers.

In attempting to assess whether selection of a particular contraceptive method identifies a group of women with a high prevalence of cervical neoplasia, we must remember that women with two or more smears had all but one of the smears taken before entry. At entry either the woman or the physician might select a contraceptive based on previous events rather than personal preference. For example, the Grady Family-

Planning Clinic receives referrals from the Grady Tumor Clinic. Often such referred women have never previously been enrolled in the Family-Planning Clinic; they are referred specifically to receive dehydromedroxyprogesterone acetate injections so that they will not become pregnant while they are having biopsies to confirm their abnormal Pap smear. Because of the above, we feel that women with one Pap smear, the one taken at the time they select their contraceptive method, give the least biased answer to the question at issue.

For women with one Pap smear, oral contraceptive choosers have *less* carcinoma in situ than do IUD choosers. We interpret this to mean that, at the time of choosing their method, oral contraceptive choosers manifest less risk of developing carcinoma in situ than do IUD choosers. In women with one Pap smear oral contraceptive choosers had a 40 per cent higher risk of developing dysplasia. In women with two or more Pap smears oral contraceptive choosers had virtually the same risk of carcinoma in situ and a 50 per cent higher risk of dysplasia than did IUD choosers. However, as discussed, the higher risk of dysplasia in this latter group does not necessarily reflect their intrinsic risk of dysplasia; instead, it may reflect patient or physician bias.

Subjects in our study have a high prevalence rate of cervical dysplasia and carcinoma in situ when compared to other large populations. For our subjects the dysplasia prevalence rate in 20- to 29-year old oral contraceptive and IUD choosers in 2.5/100; the carcinoma in situ prevalence rate in such women is 0.4/100. The prevalence rate of dysplasia in women of similar ages in other published data ranged from 1.1 to 1.6 per 100 women screened; carcinoma in situ prevalence was 0.2 to 0.4 per 100.[12–15]

Stern and associates[7] studied the association between choice of contraceptive and prevalence of cervical dysplasia in a population somewhat similar to ours with respect to demographic and contraceptive characteristics. Both studies show that dysplasia prevalence was slightly higher in oral contraceptive acceptors than IUD acceptors. The difference was not statistically significant in either study.

The group of women studied by Melamed and his colleagues[5] is dissimilar to ours with respect to demographic characteristics—their subjects were older and more parous and fewer of them were blacks than in our study. Another crucial difference is that the authors compared carcinoma in situ prevalence rates in oral contraceptive and diaphragm choosers. Finally, they reached a different conclusion: they showed a strong association of contraceptive choice and carcinoma in situ. The differences in the conclusions between the study of Melamed and associates and our study may be explained by both the demographic differences between the study populations as well as the differences in the comparison group.

These results suggest that the decision to use oral contraceptives, per se, does not identify a group of women at higher risk to develop cervical carcinoma in situ. Such a decision, may, however, identify a group with a small increased propensity for developing cervical dysplasia.

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Serum prolactin concentrations in women treated with chlormadinone acetate

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Serum prolactin concentrations were measured serially in eight normal women who used continuous chlormadinone acetate treatment (500 μg per day) for six months as part of an investigational new drug contraceptive study. Circulating prolactin was also measured in women using several additional oral contraceptive steroid preparations, including a sequential mestranol–mestranol + chlormadinone acetate regimen. No increase in basal serum prolactin concentration was produced by chlormadinone acetate treatment, either alone or when added to estrogen therapy. These results suggest that chlormadinone acetate may not alter human mammary tissue through a prolactin mechanism.

ALTHOUGH THERE IS abundant evidence that estrogens increase the basal concentrations of prolactin and enhance the serum prolactin response to various stimuli,[1] the effect of other hormonal steroids on prolactin secretion is less clear. In particular, the effect of contraceptive steroids on circulating prolactin has not been studied systematically.

There is a considerable body of evidence that prolactin is an important factor in the growth of mammary carcinoma in laboratory animals. Hypophysectomy can induce remissions of animal mammary cancer, and this effect can be neutralized by prolactin administration.[2] In addition, the inhibition of prolactin secretion with ergot alkaloids can reduce tumor growth.[3] Both hypophysectomy and L-dopa (an inhibitor of prolactin secretion) have been reported to suppress breast cancer growth in women.[4, 5] Sinha, Cooper, and Dao[9] have reported that stimulation of pituitary prolactin release by electrolytic stimulation of the hypothalamus greatly accelerated the growth rate of mammary tumors in rats, although this effect was estrogen-dependent. Because the hypophyses of dogs treated with chlormadinone acetate exhibit an increase in the number of acidophilic cells,[7] Hill and Dumas[8] have postulated that chlormadinone-induced prolactin secretion may be responsible for the increased incidence of breast nodules observed in dogs treated with this agent. However, these investigators did not measure circulating prolactin concentrations in their dogs.

The purposes of this study are: (1) to measure prolactin concentrations serially in women treated with chlormadinone acetate, a synthetic progesterone derivative; and (2) to compare these changes with those observed in women treated with other contraceptive steroids including mestranol, and mestranol in combination with chlormadinone acetate or derivatives of nortestosterone.

Materials and methods

Serum specimens were obtained after an overnight fast in ambulatory women incidental to the performance of oral glucose tolerance or intravenous arginine tolerance tests before and during contraceptive steroid treatment. The women were instructed not to eat or drink for at least 12 hours prior to the test, and each woman was seated for 15 minutes before the serum specimen was obtained. For all but six of the women, control specimens were obtained four to six weeks postpartum, and none of these subjects was

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Table I. Serum prolactin concentrations (μl-Eq/ml.) in normal women after an overnight fast during treatment with various oral contraceptive steroid preparations. Except where noted, all samples obtained on fourteenth day of monthly cycle

Contraceptive steroid	*No. of subjects*	*Duration of treatment*			
		Before	*2 Wk.*	*2.5 Mo.*	*6 Mo.*
Chlormadinone acetate	8				
Mean		51.5	30.7	32.5	22.4
(±S.D.)		(36.3)	(17.5)	(7.5)	(15.1)
Range		12-130	8-56	18-40	10-44
Norethindrone (10 mg.)	6				
Mean		83.2	76.8	—	—
(±S.D.)		(19.4)	(22.8)	—	—
Range		51-104	54-115	—	—
Mestranol (80 μg)	20				
Mean		59.6	67.9	52.3	—
(±S.D.)		(33.4)	(56.6)	(43.8)	—
Range		8-112	18-260	—	—
Mestranol (80 μg) + chlormadinone acetate	8				
Mean		61.0	79.2*	38.7*	—
(±S.D.)		(27.3)	(53.6)	(31.6)	—
Range		28-96	18-160	10-84	—
Mestranol, 100μ + norethindrone, 2 mg. or + ethynodiol diacet, 1 mg.	12				
Mean		34.6	67.7	34.8†	—
(±S.D.)		(17.3)	(29.2)	(5.2)	—
Range		18-74	34-114	30-42	—

*Samples obtained on twenty-first day of pill cycle,
†Five subjects only.

breast feeding. The remaining subjects had not been pregnant or used any hormonal steroid for at least one year prior to the study. After obtaining the control specimen, the subjects were placed on one of the following treatment regimens: chlormadinone acetate,* 500 μg per day for six months (N = 8); norethindrone, 10 mg. per day for three weeks (N = 6†); mestranol, 80 μg per day for two weeks supplemented with mestranol with chlormadinone acetate; 2 mg. per day for an additional week (C-Quens) (N = 8); or mestranol (100 μg) + norethindrone (2 mg.) (N = 6) or ethynodiol diacetate (1 mg.) (N = 6), administered in combination daily. Except for the continuous daily chlormadinone acetate treatment regimen, all steroids were administered for three weeks followed by a five to seven day interruption to allow for withdrawal menses. Additional serum specimens for prolactin determination were obtained on the fourteenth day of the first and third treatment cycle. Additional specimens were obtained on the twenty-first day of the treatment cycle for those women treated with mestranol + chlormadinone acetate sequentially and during the sixth month for the women treated with chlormadinone acetate only. All serum specimens were stored at −20° C. until assayed.

Serum prolactin concentrations were measured by a heterologous radioimmunoassay system[9, 10] using serum from a patient with a chromophobe adenoma of the pituitary as a reference standard. In our hands, 3.4 μl of this patient's serum was equivalent to 1 nanogram of human prolactin (reference standard V-L-S No. 1 from the National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health). Student's t test was used to determine the significance of difference between group means.[11]

*Supplied in tablet form by the Syntex Institute for Hormone Research. All chlormadinone acetate and C-Quens studies were performed in 1968 and 1969 prior to the FDA withdrawal of approval of chlormadinone acetate as an investigational new drug. Informed consent was obtained from all subjects who participated in the study.

†Includes the six subjects who were not postpartum.

Results

As shown in Table I, there was a wide range in the fasting serum prolactin concentrations obtained before contraceptive treatment. Presumably this was due to

Table II. Statistical analysis of data in Table I. Differences between mean serum prolactin concentrations of treatment group before and during contraceptive steroid treatment

Chlormadinone acetate	*Duration of treatment*		
	Before	*2 Wk.*	*2.5 Mo.*
Vs. norethindione	−31.7	−46.1	
Mean Δ			
p	NS	<.005	—
Vs. mestranol			
Mean Δ	−8.1	−37.2	−19.8
p	NS	NS	NS
Vs. mestranol + chlormadinone acetate			
Mean Δ	−9.5	−48.5	−6.2
p	NS	<.05	NS
Vs. mestranol + norethindrone or + ethynodiol diacet			
Mean Δ	+16.9	−37.0	−2.3
p	NS	<0.1	NS

the frequent random fluctuations in serum prolactin levels which have been previously reported.[12] Consequently, with one exception, there were no significant differences in the mean control serum prolactin concentrations in any of the treatment groups. The mean±S.D. control serum prolactin concentration of the norethindrone group (83.2 μl-Eq/ml. ± 19.4) was nearly 2 ½ times greater than pretreatment levels of the group treated with mestranol + a nortestosterone derivative (34.6 μl-Eq/ml. ± 17.3), $p < 0.05$.

During treatment with chlormadinone acetate, there was an apparent decrease in the mean serum prolactin concentration during the first, third, and sixth month of chlormadinone acetate treatment. However, none of these decreases were significant because of the large variation in the mean control values. By contrast, during the third trimester of pregnancy, when serum progesterone and estradiol concentrations are known to be very high, the mean ± SD serum concentrations of prolactin in these subjects was 456.6 ± 163.2 μl-Eq/ml.

When compared with the women using other contraceptive steroid preparations (Table II), it is evident that the women treated with chlormadinone acetate had lower mean serum prolactin concentrations (29.2 ± 16.0 μl-Eq/ml.) after two weeks' treatment than those women treated with norethindrone alone, or mestranol + a nortestosterone derivative (Tables I and II). However, these differences were not maintained during the third month of contraceptive steroid treatment. Although the mean serum prolactin concentration after three months of treatment with sequential mestranol + chlormadinone acetate was lower than after three weeks of treatment, this difference was not significant.

Comment

The present study demonstrates that the administration of a progesterone derivative to women, namely chlormadinone acetate, produces no increase in serum prolactin concentrations. Indeed, there was an apparent decrease in serum prolactin concentrations with this contraceptive steroid, but this difference was not significant because of the small number of subjects and the wide range of their values. In addition, the concurrent administration of chlormadinone acetate with a synthetic estrogen did not increase the serum concentrations of prolactin, which might be expected by treatment with the estrogen alone. These findings are similar to those of Jaffe and co-workers[13] who found no further increase in basal prolactin concentrations in women given progesterone, 10 mg. intramuscularly on the tenth day of ethinyl estradiol treatment. Similarly, Caligaris and associates[14] observed no change in serum prolactin concentrations in spayed rats after treatment with large doses of progesterone alone; however, prolactin concentrations increased after treatment with estradiol benzoate alone and in combination with progesterone. It is of some interest that Jaffe and associates[13] found an enhancement of the serum prolactin response to TRH (thyrotropin releasing hormone) after treatment with testosterone enanthate + estradiol valerate within 72 hours of delivery to suppress lactation. However, it should be noted that Buckman and Peake[1] have reported that estrogen potentiation of phenothiazine-induced prolactin secretion in women is not further enhanced by concurrent administration of a nortestosterone derivative. Thus, from the literature it appears that the estrogen component of contraceptive steroids is the agent primarily responsible for the increase in prolactin secretion in users of contraceptive steroids. This finding could not be confirmed in the present study because of the small sample size.

These findings are partially substantiated by morphologic studies. Hachmeister[15] has reported hyperplasia of pituitary prolactin cells in men treated with large doses of estrogen for carcinoma of the prostate. By contrast, Baker and co-workers[16] have observed no change in the prolactin cells in ovariectomized rats after implantation of medroxyprogesterone acetate pellets in the hypothalamus; they did observe an increase in the numbers of prolactin cells after implantation of norethynodrel or norethindrone, which they attributed to the intrinsic estrogenicity of

these two nortestosterone derivatives. Thus, the acidophilic cell hyperplasia in the pituitaries of dogs treated with chlormadinone acetate[7] appears to be a phenomenon unique to the canine species.

Finally, data currently available does not indicate that long-term treatment with a progesterone derivative produces adverse effects on the human breast. McDaniel and Pardthaisong[17] have reported no increase in the incidence of breast nodules in 1,270 women treated with depomedroxyprogesterone acetate every three months for 3 to 375 months. In addition, Douglas Ringrose[18] has observed a 50 per cent decrease in dysplasia of cells obtained from breast fluid of women treated with depomedroxyprogesterone. Moreover, in non-human primates treated with 10 to 50 times the usual human dose of contraceptive steroids, no increased incidence of mammary tumors has been observed thus far.[19]

The reason for the increased incidence of breast tumors in beagles treated with progesterone or its derivatives remains an enigma. Two possible causes for this tumor diathesis are: (1) progesterone-enhanced prolactin secretion which is peculiar to the dog, or (2) progesterone-sensitization of dog breast tissue to other endogenous carcinogens. Although chlormadinone acetate induces acidophilic cell hyperplasia in dog pituitary glands which might be associated with increased prolactin production, no measurements of circulating prolactin have been reported in the dog. Similarly, progesterone-specific receptors have been reported in 60 per cent of tumor obtained from women with breast cancer.[20] Thus, if dog mammary tissue binds progesterone or its derivatives to a much greater extent than human tissue, it is possible that the progesterone might enhance dog breast tissue growth in response to other endogenous stimuli such as estrogens, growth hormone, or prolactin. Clearly, these speculations require additional careful investigation.

In summary, the present study demonstrates no increase in circulating prolactin concentrations in women treated with chlormadinone acetate for up to six months. In conjunction with the lack of data demonstrating progesterone-induced mammary changes in women, these data provide further evidence that the dog is a questionable model for studying the carcinogenic effects of progesterone derivatives. Admittedly, the data in the present study are of limited usefulness because of the wide range of pretreatment basal prolactin values, presumably due to frequent random fluctuations of serum prolactin concentrations. However, it should be possible to confirm these results with provocative tests using secretogogues (such as phenothiazine derivatives or TRH) in women treated solely with progesterone derivatives.

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Studies on the cytodynamics of human endometrial regeneration

II. Transmission electron microscopy and histochemistry

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The morphologic observations made in this study indicate that postmenstrual endometrial resurfacing occurs by proliferation of epithelial cells originating from the stumps of residual basal glands and uterine surface lining, bordering the denuded areas, respectively. Endometrial stromal cells or glands of the residual spongiosa are not involved in uterine surface repair. The mechanism whereby the new surface develops, it is proposed, is by endomitotic division. This contention is supported by the accelerated nuclear DNA and intracytoplasmic RNA synthesis, and absence of mitosis in the newly formed surface epithelial cells. The results indicate, furthermore, that uterine surface reconstruction, which is completed by cycle day 5, is independent of ovarian hormonal stimuli, whereas further endometrial growth, connective tissue remodeling, and metabolic enzyme synthesis are influenced by the gradual increase of preovulatory estrogens.

ON THE BASIS of available morphologic data on endometrial regeneration obtained in both human subjects[1–10] and experimental animals,[11–14] it is proposed that repair of the human uterine surface[8, 9] is completed within 48 hours following the first 2 menstrual days and 60 hours after mechanically induced trauma in the rodent.[13, 14] Several sources of origin are suggested for the resurfacing epithelium. These include, in women and subhuman primates, the glands of the residual zona basalis,[3–5, 7, 8] secretory glands of the residual spongiosa,[9] and endometrial stromal cells[1, 2, 6, 10]; in the rabbit, the intact endometrial surface bordering the denuded area[13, 14]; in the mouse,[11] the peritoneal cells migrating through the oviducts. It is suggested, furthermore, that during endometrial resurfacing the accelerated synthesis of collagen contributes to the expansion of the uterine mucosa.[9, 10]

Our recent SEM study[15] on human endometrium during the process of repair indicates a dual origin of the developing surface epithelium: primarily, from the free gland stumps of the residual zona basalis and, in a lesser degree, from the persisting lining epithelium of the lower uterine and peritubal (cornual) regions, adjacent to the denuded mucosa. In agreement with previous observations on human endometrial regeneration,[8, 9] surface epithelial restoration begins in between cycle days 2 and 3 and ends in between cycle days 4 and 5. The process of regeneration is presumably initiated by loss of endometrial tissue including the spongiosa as reconstruction begins only in areas in which the basal gland mouths are freed from the overlying, degenerated tissue.[15] The mechanism of surface re-epithelialization occurs by a simultaneous and progressive epithelial outgrowth of the free ends of basal glands and by ingrowth of the intact, peripheral surface membrane bordering the denuded basalis. Subsequently, interanastomoses are established between neighboring epithelial proliferations leading ultimately to the obliteration of the denuded postmenstrual uterine mucosa.[15]

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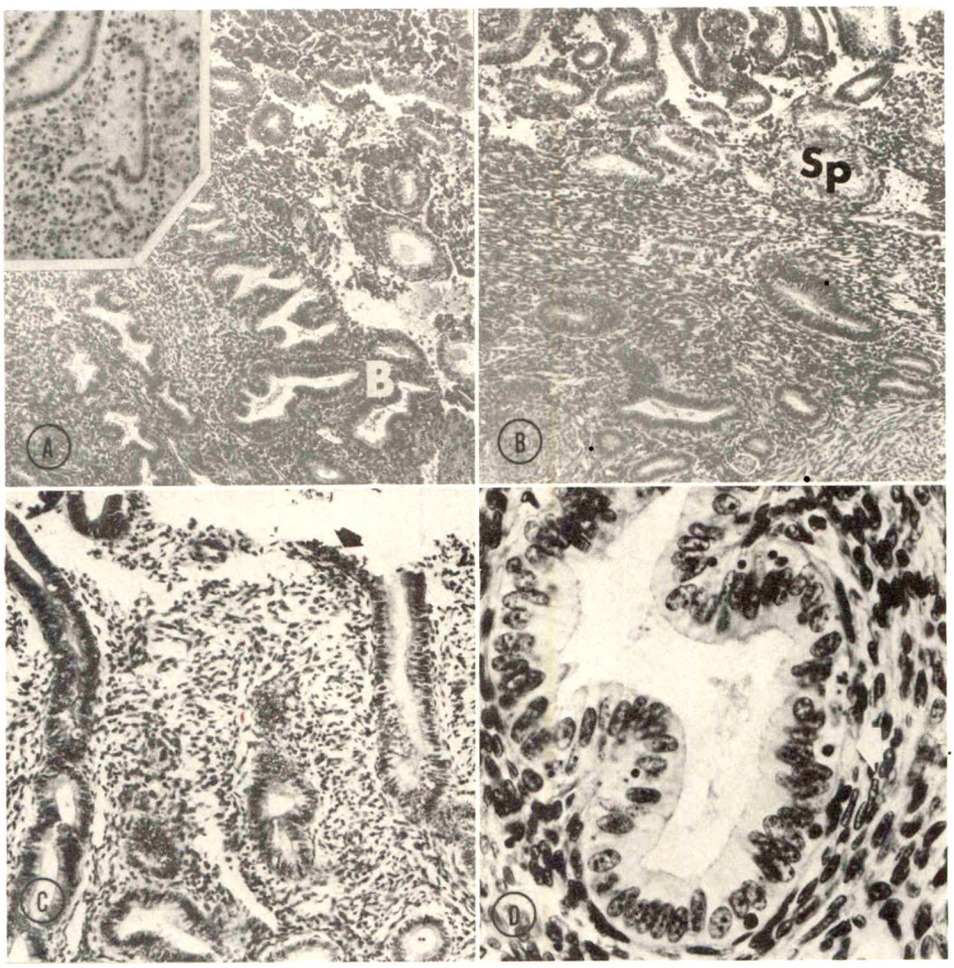


Fig. 1. *A*, Cycle day 2. Disruption of spongiosa (right) extends to the basalis *(B)*, flanked by as yet intact endometrium. The latter has tortuous glands including their basal portion, lined by cuboidal cells, and contains intraluminal secretions. (Hematoxylin and eosin stain; ×70.) *Insert:* Cycle day 1. Detailed view of ruptured and collapsed glands of the upper spongiosa intermingling with degenerated predecidual cells and erythroleukocytic debris. (Hematoxylin and eosin stain; ×90.) *B*, Cycle day 2. The spongiosa *(Sp)* just above the basalis consists of disintegrating glandular and stromal elements which seem to dissociate from the underlying zona basalis. (Hematoxylin and eosin stain; ×80.) *C*, Cycle day 3. Early epithelial extension (arrow) of basal gland origin spreads out over the adjacent stroma. (Hematoxylin and eosin stain of rehydrated SEM specimen[15]; ×160.) *D*, Cycle day 3. A basal gland is shown with "poly-dust" (arrow). Note the compact stroma, made of immature spindle-shaped fibroblasts. (Hematoxylin and eosin stain; ×850.)

Despite the presently available information on endometrial regeneration, there remain several features to be further examined and elucidated. These relate to (1) the fine structural characteristics of the proliferating surface and residual basal glandular epithelium and (2) the role of stromal cell growth and collagen synthesis in the expansion of postmenstrual endometrium.

Materials and methods

For light and electron microscopy, endometrial specimens were obtained from 24 normal menstruating women (aged 22 to 41 years) on cycle days 1 to 6 exclusively and on cycle days 9 and 10. The selection of patients, date of recovery, and processing of specimens are described in detail in our previous communication on endometrial regeneration.[15]

For electron microscopy, small blocks of endometrium including the basalis were recovered from representative areas of the endometrial cavity.[15] Tissues were fixed in 3 per cent glutaraldehyde in 0.1M cocodylate buffered at pH 7.2. Postfixation was carried out in 1 per cent osmium tetroxide and the tissues were embedded in Epon. Thin sections were stained with 2

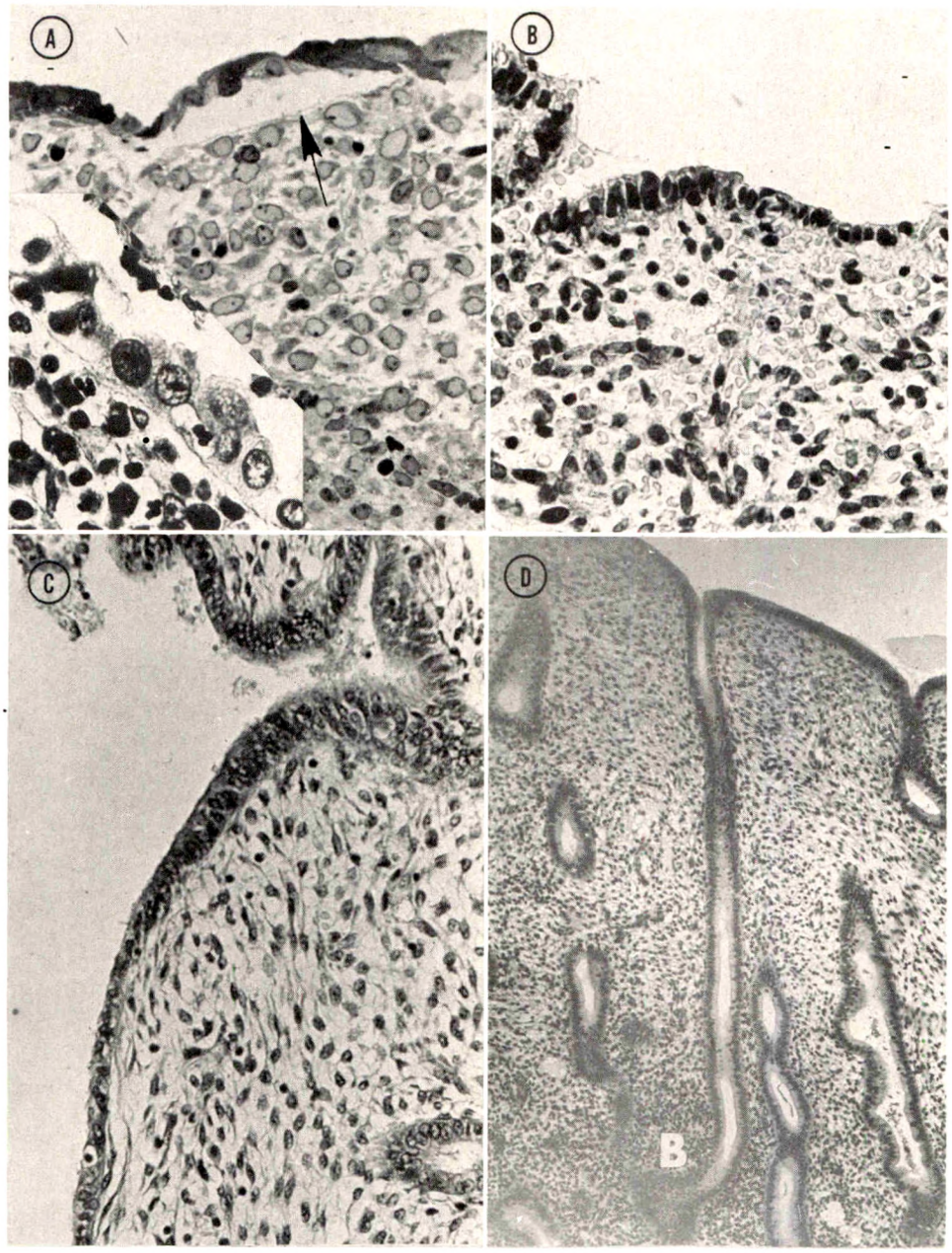


Fig. 2. *A*, Cycle day 3. The developing surface epithelial cells are flattened with pleomorphic nuclei and nucleoli. Due to the fragility of resurfacing epithelium, it is often artificially detached from the underlying stroma or basal lamina (arrow). (Thick section; toluidine blue stain; ×850.) *Insert:* Higher magnification of regenerating surface epithelial cells supported by a well-developed basal lamina. (Hematoxylin and eosin stain; ×1,200.) *B*, Cycle day 4. Characteristically, the advancing portion of the newly formed surface epithelium is thinner (right) than near the gland mouths (left). The subjacent stroma is loose, contains extravasated red blood cells and immature stromal cells with scant cytoplasm. (Hematoxylin and eosin stain; ×750.) *C*, Cycle day 5. Generation of a new surface layer is completed. The gradual enlargment of epithelial surface cells toward basal gland mouths reflects their progressive maturation and basal gland neck origin. The stroma remains myxoid with stellate fibroblasts, devoid of mitosis. (Hematoxylin and eosin stain; ×760.) *D*, Cycle day 6. The columnar surface epithelium is interconnected by a proliferating gland of basal zone origin *(B)*. There are numerous mitotic figures in both glandular and stromal cells. (Hematoxylin and eosin stain; ×400.)

per cent uranyl acetate and Reynold's lead citrate and examined in a Hitashi, H.S.-8.2 electron microscope at 50 KV.

For histochemistry, portions of tissues of each block used for SEM,[15] light and transmission electron microscopy were fixed in 10 per cent neutral formalin for the detection of acid phosphatase and in absolute acetone at −5° C. for alkaline phosphatase. After dehydration and embedding, acid phosphatase was demonstrated by incubating tissues for 2 hours in diazotate of O-aminoazotoluene (in GBC salt)[16] and alkaline phosphatase by using the calcium-cobalt method of Gomori[17] with an incubation time of 5 hours at 37°C. For neutral mucins, periodic acid–Schiff with diastase digestion was used; for acid mucins, high-iron diamine-alcian blue at pH 2.5; for collagen and reticulin; Masson's trichrome and Wilder's reticulin. Tissues for these stains were fixed in Bouin's solution.

Results

Light microscopy. Specimens representing the first 2 days of the cycle (Fig. 1, *A* and *B*) have fields of mucosal breakdown in which predecidual cells aggregate and degenerate, glands collapse, and the edematous extracellular space is infiltrated by erythroleukocytic elements. On cycle day 1, these changes are predominant in the subepithelial compacta layer with focal encroachment of the spongiosa. On cycle day 2, the process of dissolution involves most of the spongy layer with extension to the basal zone. In this region, the secretory glands are separated from their supportive stroma and seemingly float within the edematous extracellular space (Fig. 1, *B*). Acid phosphatase with a perinuclear distribution is prominent in glandular, endothelial, and predecidual cells of the upper endometrial layers. The as yet uninvolved glandular units are complexly convoluted with papillary infoldings including their basal portions and the lumen contains PAS-positive–diastase-resistant secretions with greater concentration in the glands of the zona basalis (Fig. 1, *A*). Scant alkaline phosphatase is detectable within intraluminal secretions. The glandular epithelium of the compacta and upper spongiosa is generally low cuboidal due to "secretory exhaustion" and low-columnar in the lower spongy and basal layers (Fig. 1, *A*). Apocrine secretory activity and accumulation of apical, intracytoplasmic mucopolysaccharides as seen in glands of the spongiosa are not observed in basal glands. The zona basalis has a compact stroma which remains intact during the entire menstrual period. It is made of closely spaced spindle-shaped fibroblasts with scant cytoplasm and hyperchromatic nuclei devoid of mitosis (Fig. 1, *B* to *D*). Wilder's reticulum and Masson's trichrome stains demonstrate abundant reticulum and collagen fibers in the basal area and fragmentation in the overlying spongiosa and compacta.

Most uteri of the third day of the cycle have a denuded basal layer with ragged and often hemorrhagic surface onto which open numerous basal glands (Fig. 1, *C*). The lining epithelium is most often low-columnar with round to oval nuclei located near or on the basal lamina and nucleoli are prominent (Fig. 1, *C*). The abundant cytoplasm is finely granular and often contains the characteristic "poly-dust" in a subnuclear location (Fig. 1, *D*). The latter is believed to represent phagocytosed nuclear debris of polymorphonuclear origin and is a constant feature in cyclic menstrual endometrium. The presence of faint acid phosphatase in these epithelial regions suggests that the incorporated emperiopoietic cells undergo hydrolytic enzyme degradation. The gland stumps are usually lined by PAS-negative, cuboidal epithelium with extensions onto the denuded surface (Fig. 1, *C*). The developing surface epithelium is low-cuboidal and demonstrates nuclear enlargement with prominent nucleoli (Fig. 2, *A*). The cytoplasm is often strongly acidophilic and varies in shape and size. The epithelial cells which are located at the distal end of the advancing surface lining are flat to spindle-shaped, devoid of division figures. With the aid of the PAS stain a clear demarcation between the superficial epithelial and underlying stromal cells is obtained, as evidenced by the presence of a well-defined basal lamina (Fig. 2, *A* and *B*). No evidence of acid or alkaline phosphatase activity is noted in the regenerating surface epithelium.

The residual surface lining of lower uterine segment and peritubal (cornual) regions is made of well-preserved, low-to-tall-columnar cells alternating with numerous ciliated cells.[15] It becomes low-cuboidal to flattened, however, as it spreads onto the neighboring stroma of the zona basalis. A basal lamina is uniformly demonstrated running parallel to the migrating surface epithelium.

Most uteri removed during the fourth day of the menstrual cycle have a fully re-epithelialized surface (Fig. 2, *B*). The lining epithelium is low-columnar near the glandular openings with occasional ciliated cells and cuboidal to flattened in the interglandular areas. The enlarged nuclei contain anisonucleolosis and mitosis is absent. The basal glandular epithelium exhibits similar morphologic characteristics to those observed during the previous day. Unlike the rather dense stromal surface layer during cycle day 3, on day 4 the subepithelial zone is myxoid and composed of

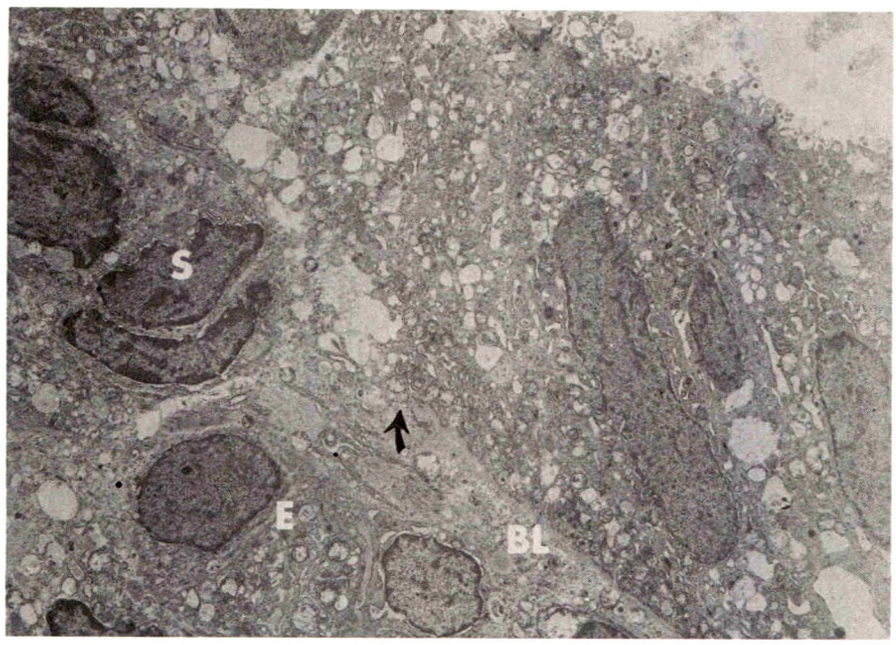


Fig. 3. Cycle day 3; residual spongiosa. The glandular, stromal *(S)* and endothelial *(E)* cells are degenerated with vaculated organelles, retracted electron-dense nuclei, and ruptured glandular (arrow) basal lamina *(BL)*. (×6,250.)

immature, stellate fibroblasts without mitosis (Fig. 2, *B*). Neither alkaline nor acid phosphatase is observed in the basal glandular, stromal, or endothelial cells during the third and fourth days of the cycle.

Endometria obtained on cycle days 5 and 6 display a dramatic change in the morphology of both the glandular apparatus and stroma of the zona basalis (Fig. 2, *C* and *D*). The attenuated cryptic cells of premenstrual, menstrual, and regenerative periods are suddenly transformed into tall-columnar cells with nuclear pseudostratification. The mid and upper glandular segments are lined by columnar and low-columnar cells, respectively. Mitoses and ciliated cells are seen at all glandular levels, including the deep crypts and surface epithelium. The maximum mitotic and ciliogenic activity, however, is observed in the upper half portion of the glands and circumosteal regions (Fig. 2, *D*). The endometrial glandular and surface cells contain scant acid phosphatase activity, chiefly in a supranuclear location, and alkaline phosphatase in the apex and surface plasma membranes. During the same period, the stromal cells remain immature with poorly developed cytoplasm, devoid of metabolic enzymes, and the nuclei and the nucleoli are prominent (Fig. 2, *C*). Mitotic figures are occasionally encountered. The loose and myxoid extracellular space is faintly alcian blue–colloid iron positive, indicating the presence of acid mucopolysaccharides. Due to epithelial proliferation, the glands of basal zone origin increase in length and number and are often seen interconnecting the newly formed surface epithelium with the basal crypts (Fig. 2, *D*).

Specimens of days 9 to 10 of the cycle contain morphologic evidence of further glandular-stromal cell proliferation and maturation. Indeed, mitosis is increased in epithelial and stromal cells, as is surface epithelial and glandular ciliogenesis. In addition, the glands undergo multibranching and tortuosity, and synthesis of extracellular collagen, as evidenced by the Masson's trichrome and alcian blue–colloid iron stains, is observed. Similarly, reaction products of both alkaline and acid phosphatase are localized in a comparatively greater amount and in a greater number of glandular cells than on cycle days 4 and 5.

Electron microscopy. In between cycle days 1 and 3, the glandular, predecidual, and vascular compartments of the compacta and spongiosa, including its suprabasal portion, feature severe degenerative changes reflecting irreversible cell injury (Fig. 3). These consist in pyknosis, karyorrhexis and karyolysis, organellar vacuolization, fragmentation of plasma membranes, loss of desmosomal belts, and rupture of glandular and endothelial basal lamina. The changes are produced upon the destructive effect of hydrolytic lysosomal enzymes such as acid phosphatase and possibly cathepsin-D.[18] The enzymes are thought to be

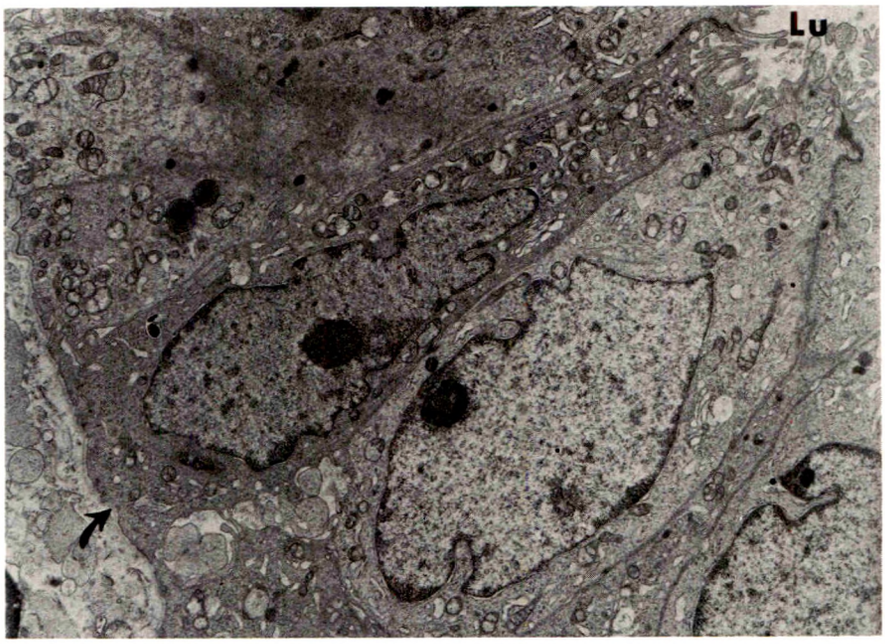


Fig. 4. Cycle day 3; basal layer. Electron micrograph of basal gland neck cells. The cytoplasmic organelles, such as mitochrondria and free and bound ribosomes, are well developed, whereas the Golgi is inconspicuous and secretory activity is absent. The nuclei are elongated with compact chromatin and nucleoli are prominent. *Lu*, Glandular lumen; arrow, basal lamina. (×8,800.)

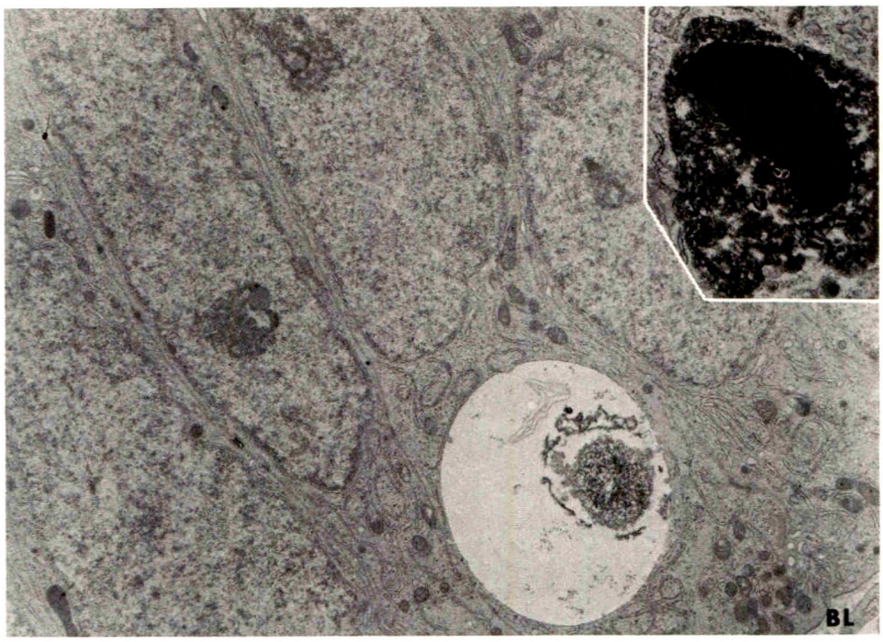


Fig. 5. Cycle day 3; basal layer. A prominent, subnuclear vacuole within a basal gland crypt cell contains electron-dense, granuloflocculent material representing cellular debris in an advanced stage of dissolution. The structure corresponds to the so-called "poly-dust" seen in Fig. 1, *D. BL,* Basal lamina. (×8,800.) *Insert:* Electron micrograph of a phagocytosed, intraepithelial cell in an earlier stage of cytonuclear digestion. The presence of a single, dense nuclear material favors its mono- rather than polymorphonuclear cell origin. (×16,000.)

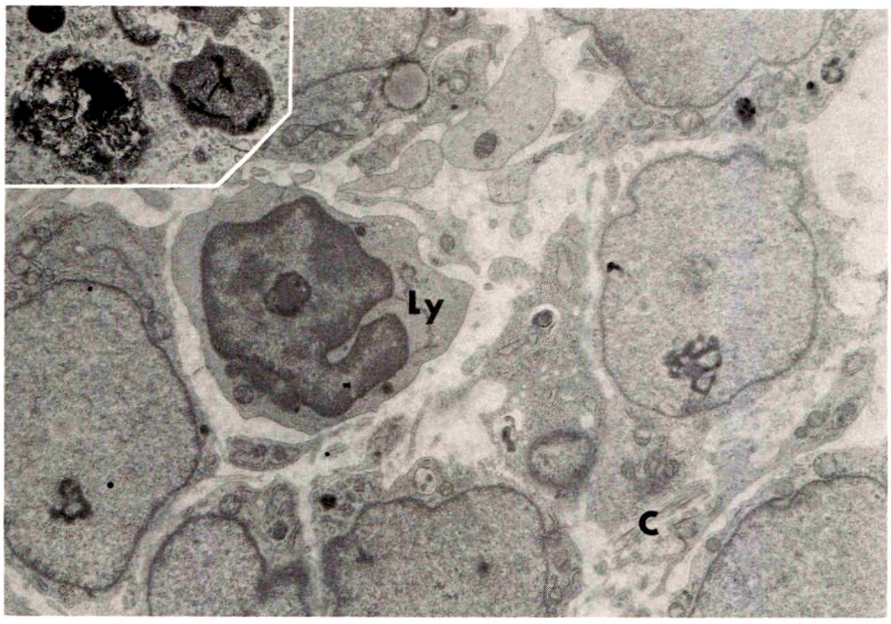


Fig. 6. Cycle day 3; basal layer. Stromal cells near the endomyometrial junction are shown. They are close to each other, have an immature appearance with finger-like cytoplasmic processes and prominent nuclei with euchromatin pattern. The cytoplasm of most cells is scant, containing few organelles, whereas others have better organellar constitution, involved in collagen *(C)* synthesis. A basal lamina is absent from endometrial fibroblasts. *Ly;* Lymphocyte. (×8,800.) *Insert:* Membrane-bound cytophagolysosomal bodies with partly digested material, presumably of inflammatory cell origin, are seen. These structures are often found within stromal cells near the surface of the residual basal layer. (×16,000.)

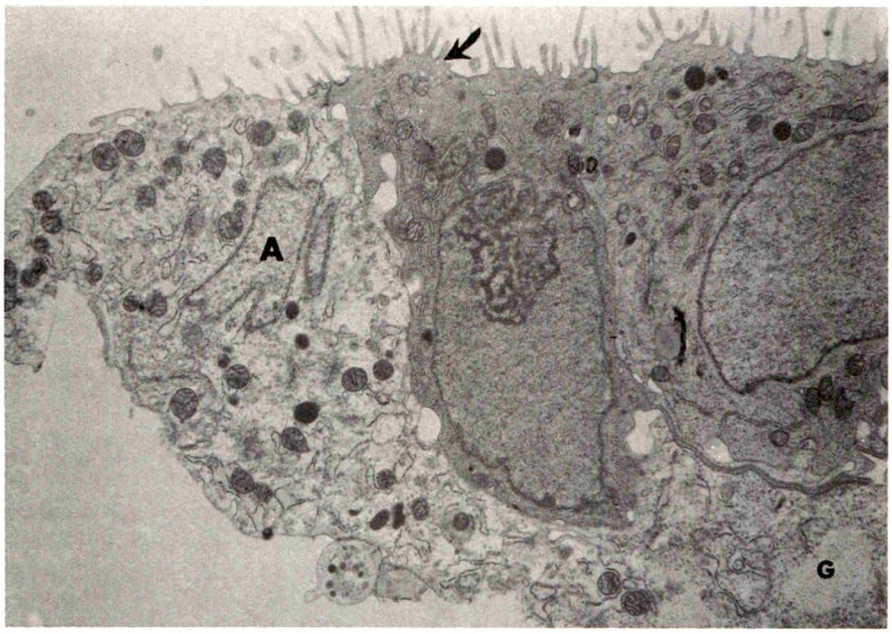


Fig. 7. Cycle day 3; regenerating surface epithelium. Electron micrograph illustrating the most advanced portion of developing surface epithelium. The cells are cuboidal, well preserved, and contain abundant organelles, collections of glycogen granules *(G),* and prominent nuclei and nucleoli. Surface microvilli are associated with pinocytic vesicles (arrow) and the lateral cell membranes are in close apposition. These features are consistent with active cell metabolism and nuclear DNA synthesis, promoting cell growth. The most distal cell of the advancing epithelium *(A)* has artificially been detached from the underlying stroma. (×8,800.)

released following the labilization of lysosomal membranes by endogenous progesterone.[18] Ultimately, cellular collapse and dehiscence ensue, accompanied by the appearance of erythroleukocytic elements and vascular occlusion. Unlike the glands of the spongiosa, the residual basal glands remain well preserved and cellular integrity is maintained by desmosomes (Fig. 4). The epithelium lining the neck and lower glandular regions contains numerous mitochondria and free and bound ribosomes. The Golgi, however, is poorly developed and lysosomes and glycogen particles are only rarely seen. Apocrine secretory activity is not seen in basal gland cells. Most nuclei are large, with evenly distributed nuclear chromatin and the nucleoli display perinucleolar chromatin condensation. The epithelial cells of basal gland crypts have similar nuclear, nucleolar, and cytoplasmic architecture to that described for cells of the upper glandular levels. Many cryptic cells have prominent membrane-bound vacuoles in a subnuclear location, in which electron-dense material of all stages of degradation is seen (Fig. 5). These structures represent phagocytosed nuclear material of inflammatory cell origin and correspond to the so-called "poly-dust" seen at the light microscopic level. With the TEM, however, phagocytosed intraepithelial cells appear to be of mononuclear lymphocytic rather than polymorphonuclear cell origin. The migrating cells are seen within the intraepithelial and extracellular space and within intracellular phagosomes. Most stromal cells of the lower portion of the zona basalis have scant and poorly equipped cytoplasm and large spindle-shaped nuclei with prominent nucleoli resembling immature fibroblasts (Fig. 6). Others are engaged in active fibrillogenesis, indicated by the well-developed Golgi-GER-mitochondria complex associated with intracytoplasmic tropocollagen and extracellular collagen fibres. By contrast, stromal cells near the denuded surface and upper levels of the residual basal zone are involved in active phagocytosis and digestion of menstrual cellular debris and collagen. Many of these cells have a close resemblance to macrophages and contain lysosomes, residual bodies, and cytophagolysosomes (Fig. 6, insert).

The resurfacing epithelium during the early phases of its development (cycle day 3) demonstrates nuclear enlargement, anisonucleolosis, with perinucleolar chromatin concentration (Figs. 7 and 8). Mitotic activity, however, is uniformly absent in these as well as in the underlying glandular cells. The cytoplasm is well preserved, flattened to low-cuboidal, and is rich in free ribosomes, GER, mitochondria, and Golgi. Several intraepithelial emperiopoietic lymphocytes are observed within the intercellular spaces, just above the basal lamina. Due to the fragility of the newly formed

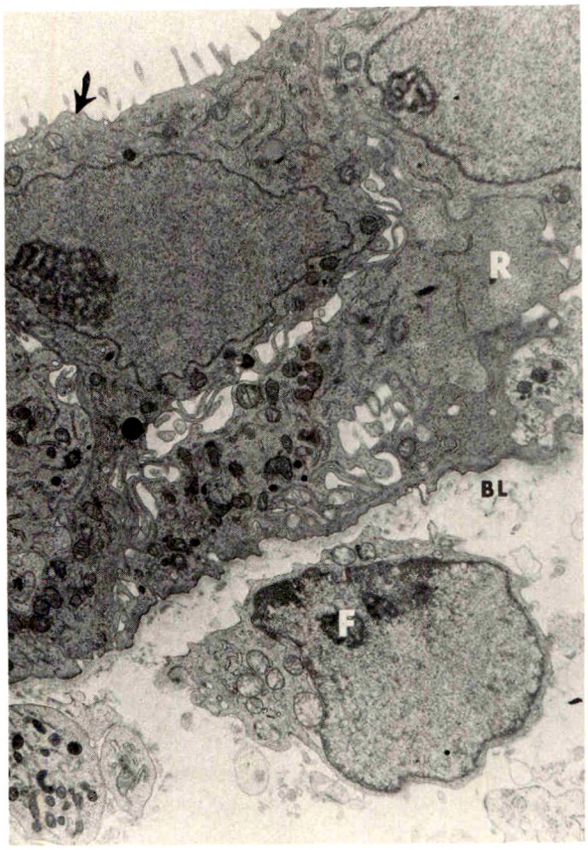


Fig. 8. Cycle day 3, regenerating surface epithelium. Surface epithelial cells near glandular openings are packed with free ribosomes *(R)*, a feature which is responsible for their dark, acidophilic appearance on histology. The nuclei have evenly distributed chromatin and there is anisonucleolosis. The presence of numerous surface pinocytic vesicles (arrow) and dilated intercellular spaces indicate that the surface lining cells are involved in active transport of water, electrolytes and other cellular nutrients. A basal lamina *(BL)* separates the epithelial cells from the subjacent fibroblasts *(F)*. (×8,800.)

epithelium, its advancing end was often detached from the underlying tissue during the preparative procedures (Fig. 7). The most distal surface epithelial cell has one of its lateral boundary membranes freely exposed to the stroma or the uterine cavity and the other, tightly adherent to its neighbour (Fig. 7). A well-defined basal lamina separates the developing uterine lining from the subjacent stromal cells (Fig. 8). Cells intermediate between stromal and epithelial units or squamoid arrangement of stromal cells[10] are not encountered. Similar structural alterations are seen in the developing surface epithelium of LUS and peritubal origin.

On cycle day 4, the surface epithelial cells are tall-cuboidal to low-columnar with euchromatic nuclei, and the cytoplasm is packed with free ribosomes (Fig. 9). There is supranuclear accumulation of Golgi, GER,

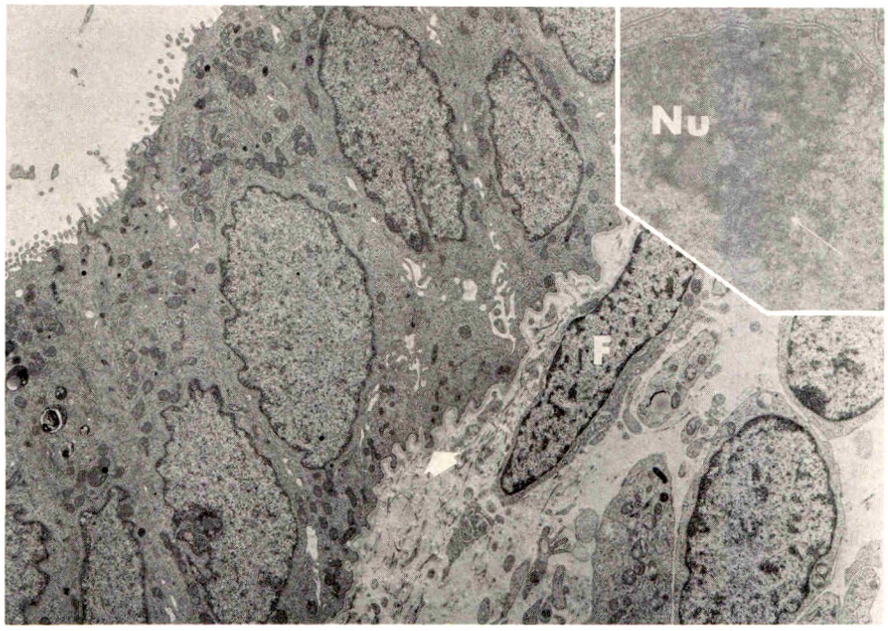


Fig. 9. Cycle day 4; surface epithelium. In general, surface epithelial cells are comparatively better developed than on cycle day 3, with supranuclear aggregates of mitochondria associated with granular ER and Golgi. Free ribosomes are abundant and dispersed throughout the cytoplasm. The cells produce a well-developed basal lamina (arrow) and the subepithelial stromal cells *(F)* resemble inactive, immature fibroblasts. (×6,250.) *Insert:* Higher magnification of a prominent epithelial nucleolar body *(Nu)* with conspicuous perinucleolar chromatin (arrow), consistent with accelerated nucleoprotein synthesis. (×18,800.)

and mitochondria. The surface microvilli, although short, are more numerous than on cycle day 3. Cells with ciliary apparatuses are occasionally seen near or within glandular ostia. Stromal cells resemble immature fibroblasts with spindle-shaped nuclei and scant, organelle-poor cytoplasm. Mitotic activity is absent and fibrillogenesis is negligable. As a result, the extracellular space beneath—and at a distance from—the surface epithelium is poor in collagen (Fig. 9).

In concordance with our histologic observations, on cycle days 5 and 6, a dramatic metamorphosis takes place in the glandular and surface epithelium as well as in stromal cell (Fig. 10). In the former, there is a considerable increase in the number of organelles, mitosis, ciliary shafts, and surface microvilli. Clusters of mitochondria are closely associated with GER, active Golgi, and free ribosomes (Fig. 11). A few membrane-bound, electron-dense primary lysosomes and bundles of microfilaments become apparent. The stromal cells have more abundant cytoplasm than on cycle day 4, with prominent nuclei and stellate or pseudopodial cytoplasmic processes (Fig. 12). Although the cells possess the necessary organelles for active fibrillogenesis, including GER, free ribosomes, Golgi, and mitochondria, synthesis of intracellular tropocollagen is minimal. The capillary endothelium features well-developed cytoplasmic organization and is particularly rich in residual bodies (Fig. 12, insert).

In between cycle days 9 and 10, the lining cells of glands and uterine surface (Fig. 13) are uniformly tall-columnar with centrally located nuclei, evenly distributed nuclear chromatin, and a large nucleolus. Many cells display mitotic divisions. The organelles are further increased in number and many mitochondria are enwrapped by parallel membranes of GER. The supranuclear Golgi is surrounded by numerous peripheral secretory vesicles and primary lysosomal bodies. Giant cytophagolysosomes are absent. An increase in RNA synthesis is reflected by the abundance of free ribosomes distributed throughout the cytoplasmic matrix. In contrast to the limited number and distribution of ciliated cells of previous days, during the midproliferative phase of the cycle, they are seen in all stages of development throughout the epithelial surface membrane (Fig. 13) and in subepithelial glandular units. Stromal cells with numerous mitoses resemble mature fibroblasts (Fig. 14). Indeed, organelles are involved in active production of collagen precursors, tropocollagen fibers. The latter are found in both cytoplasm and extracellular space, near the

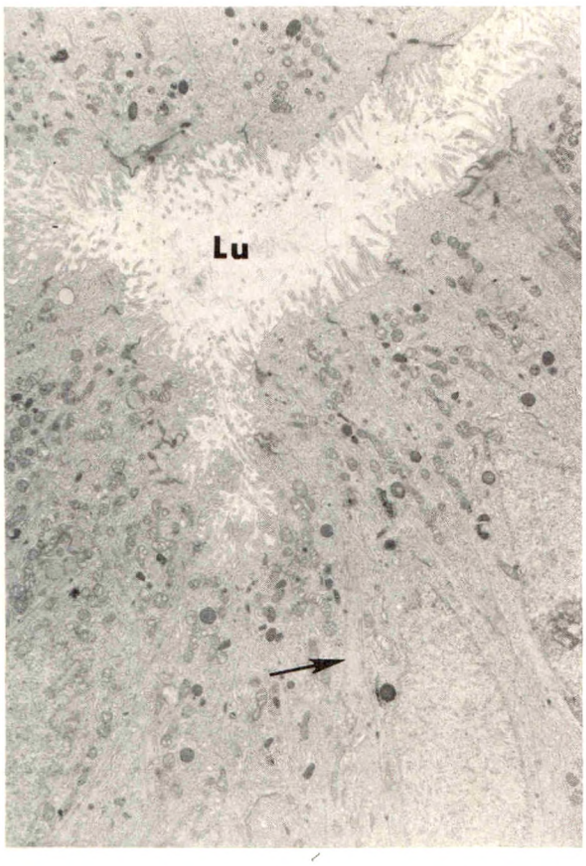


Fig. 10. Cycle day 5; basal gland neck region. There is a spectacular increase in the number of organelles in a supranuclear position, suggesting the beginning of active protein synthesis. The luminal *(Lu)* microvilli are considerably taller and more numerous than on cycle days 3 to 4. Primary lysosomes are infrequent and bundles of perinuclear microfilaments (arrow) are seen. (×6,250.)

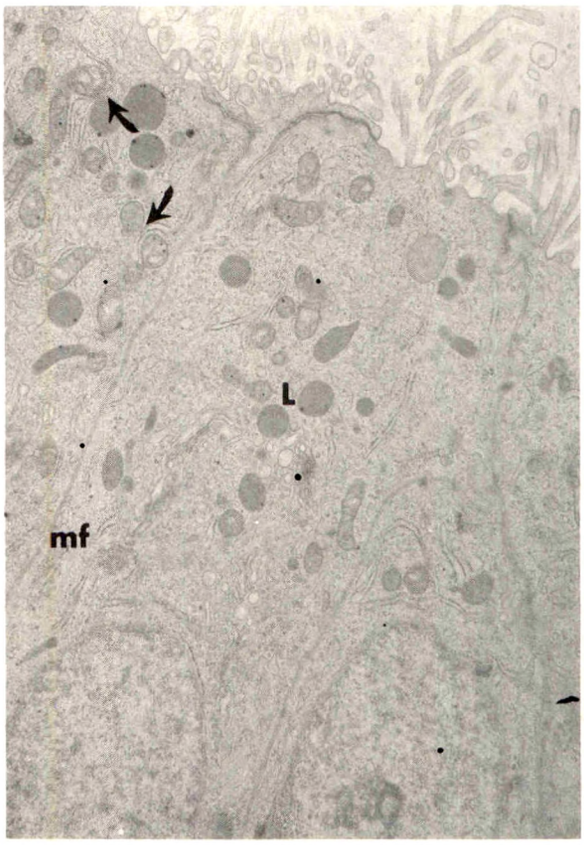


Fig. 11. Cycle day 5; basal gland neck region. Detailed view of the supranuclear region of glandular cells. The well-developed Golgi is associated with secretory vesicles and primary lysosomes *(L)*. Note the close spatial relationship between the mitochondria and parallel membranes of granular ER (arrows). *Mf,* microfilaments. (×16,000.)

plasma membrane. Concomitant with stromal cell proliferation and fibrillogenesis, the arteriolar endothelium becomes better equipped with organelles and primary lysosomes. These features together with increased mitotic activity are consistent with vascular growth, which is programmed to provide adequate blood supply to the proliferating endometrium.

Comment

The present fine-structural study of the uterine surface epithelium during the early phases of its development demonstrates evidence of nuclear enlargement with euchromatin nuclear pattern, anisonucleolosis, accumulation of perinucleolar chromatin, and abundant cytoplasmic organelles. These features are consistent with active nuclear DNA and intracytoplasmic RNA synthesis. In accordance with previous histologic observations on human[9, 10] and experimental[13, 14] endometrial regeneration, mitotic activity is uniformly absent in the resurfacing epithelium as well as in the underlying glandular and stromal cells. In view of these findings, it is possible that nucleomegaly with hyperchromasia in the developing surface epithelial cells reflects nuclear polyploidization. Although measurements of DNA values in regenerating endometrial cells are not available, similar nuclear alterations were described in cells with polyploidy, associated with hypertrophic or hyperplastic conditions.[19] In these situations the cells are required to function at a considerably high metabolic level. By doing so, i.e., multiplying nuclear DNA content, the cells adapt themselves to their environment. The mechanisms responsible for polyploidization (generation of regular multiples of the diploid quantity of DNA) are either failure of cytokinesis following mitotic division (endomitosis) or fusion of interphase nuclei during

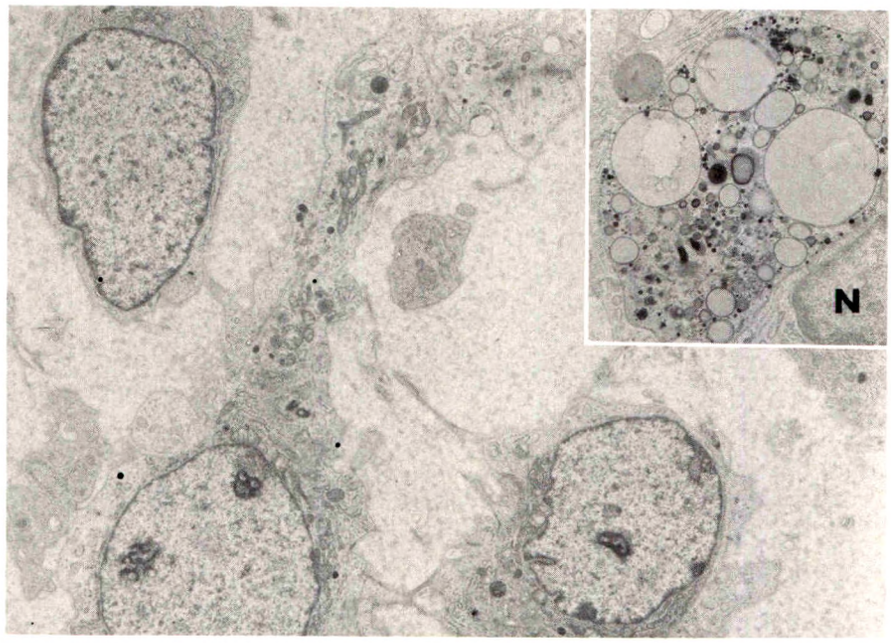


Fig. 12. Cycle day 5; stroma. The stellate stromal cells demonstrate organellar differentiation but fibrillogenesis is not yet apparent. The extracellular space is loose, containing amorphous, flocculent material. Collagen fibers are inconspicuous. (×6,250.) *Insert:* Portion of an arteriolar endothelial cell with a giant, perinuclear *(N)* residual body filled with lipid granules. (×15,000.)

mitosis. The precise reason for such cellular response in the resurfacing endometrial cells, however, is presently not apparent.

Cells located at the most distal end of the advancing surface epithelium possess desmosomal connections with their neighbors, and a well-defined basal lamina separates them from the supportive stroma. The basal lamina is produced by epithelial cells and is made of a network of fine microfibriles resembling trophocollagen. It provides support for cells, control of permeability, and cell orientation. These functions are designed to protect and enhance the development of a new surface epithelium. Consequently, epithelial regeneration may be facilitated by persistence of the basal lamina. In the present investigation, persisting and denuded glandular basal membranes were not seen, but it is conceivable that such structures remain at the site of cleavage between the degenerative spongiosa and residual basal glands providing seat of, and accelerating cellular growth.

The finding of well-preserved, metabolically active surface epithelial cells in direct continuity with the mouths of residual basal glands, and their gradual maturation toward the gland neck region, lend support for regarding the basal glands as the major source of the regenerating uterine surface membrane.[15] Identical morphologic characteristics are noted in the developing surface lining of lower uterine segment and peritubal "cornual" regions, which represent another site of origin for the resurfacing epithelium. The latter pathway was described to be the primary responsible for uterine surface reconstruction following trauma in the rabbit,[13, 14] but in the human subject it plays a comparatively less important role.

In the present study we found no ultrastructural evidence for metaplastic transformation of stromal cells into surface epithelial cells.[10] The so-called "indifferent cells at the stromoepithelial border," which were suggested to play an active role in surface regeneration,[10] are identified with the TEM to be migrating, intraepithelial lymphocytes. Their strong acridine orange fluorescence[10] supports this contention, as lymphocytes are known to be rich in nuclear DNA.

The concept of transformation of residual secretory glands of the spongiosa into a new epithelial surface[9] is not substantiated by our ultrastructural and histochemical studies. Indeed, both glandular and stromal cells of the spongy layer near the residual basalis (Figs. 1, *B*, and 3), feature severe cytonuclear degeneration, incompatible with cell survival. By contrast, the lining cells of the residual basal glands remain intact. Their cytonuclear organization, namely abundant free and bound ribosomes, mitochondria, and DNA-rich nuclei,

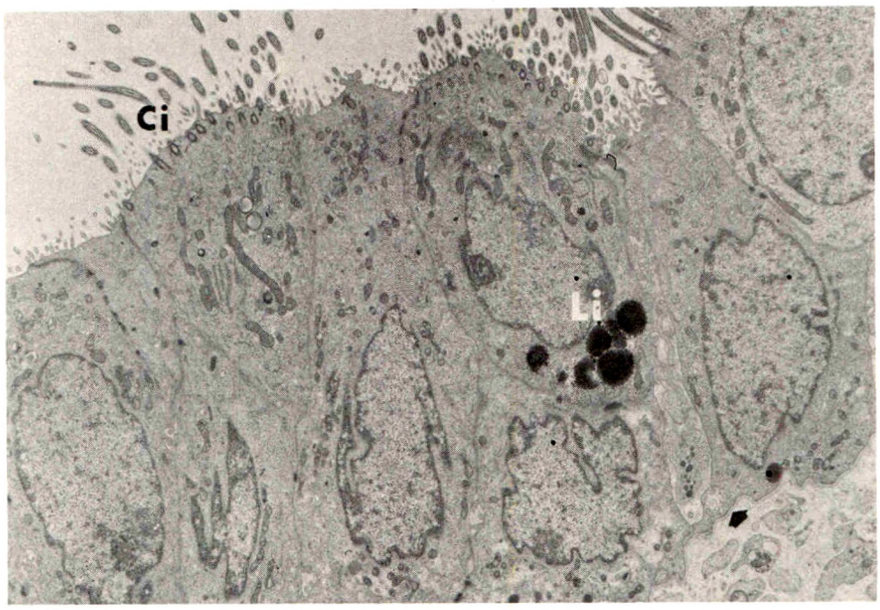


Fig. 13. Cycle day 9; surface epithelium. During the midproliferative phase of the cycle, increased protein synthesis, mitotic activity, and generation of ciliary shafts *(Ci)* are the dominant features. Lipid bodies *(Li)* are surrounded by glycogen granules. Arrow, Basal lamina. (×6,250.)

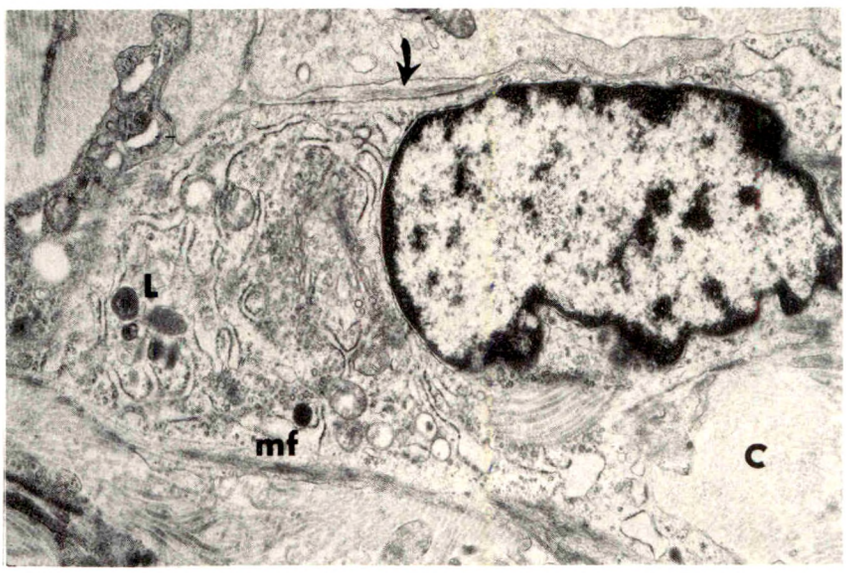


Fig. 14. Cycle day 9; stroma. The immature stromal cells of the early proliferative period are suddenly transformed into mature fibroblasts, reflected by the striking increase in cytoplasmic organelles and the appearance of peripheral, intracytoplasmic tropocollagen fibers *(mf)*. These, by focal cytoplasmic dissolution are deposited (arrow) in the extracellular space where their polymarization results in the formation of pre- and mature collagen fibers *(C)*. *L*, Primary lysosomes. (×14,000.)

are adequate for providing site of origin for the resurfacing epithelium. These cellular characteristics manifest during the second half of the luteal phase of the cycle and occur parallel to those observed in glands of the spongiosa and compacta layers. They are, however, comparatively less conspicuous[20] and secretory activity is absent. The above structural alterations are likely to represent a limited (abortive) metabolic expression, which probably reflects the relative insensitivity of basal glands to hormonal stimuli.

During the menstrual and regenerative periods, most endometrial stromal cells of the basalis resemble immature fibroblasts, devoid of mitosis and fibrillogenic activity. These observations suggest that during the regenerative period, cellular activity is focused on the reconstruction of the uterine surface epithelium rather than to stromal expansion.

Coinciding with the completion of surface epithelial repair by cycle day 5, a dramatic metamorphosis takes place in all endometrial compartments. The glandular epithelium undergoes hypertrophy; the GER-Golgi-mitochrondria complex and free ribosomes, hyperplasia. Both alkaline and acid phosphatase appear, concentrated in the microvillous surface and well-developed Golgi,[18] respectively. Mitosis and ciliogenesis are seen at all levels of the glandular apparatus, with a maximum activity in the upper half segment and periosteal surface epithelium. Similar structural modifications are seen in endometrial fibroblasts and arteriolar endothelium, including the appearance of metabolic, hydrolytic enzymes. On a morphologic basis, the development of cytoplasmic organelles, increased mitotic and ciliogenic activity, synthesis of alkaline and acid phosphatase in epithelial cells, and fibrillogenesis by stromal fibroblasts indicate the stimulatory effect of estradiol-17β.[21] It is significant that despite profound cellular remodeling, whereby cells acquire all the features of actively synthesizing units, production of glandular secretions and extracellular stromal collagen is not yet initiated. The results therefore seem to indicate that during the immediate postregenerative period (cycle days 5 and 6), cell organization, mediated by the ovarian estrogens, is selectively programmed for rapid epithelial and stromal cell growth, providing for the initial expansion of the uterine mucosa. Deposition of extracellular collagen as a contributor to endometrial growth during this period of the menstrual cycle is negligible.

While elaboration and expulsion of glandular secretory products do not occur before the time of ovulation,[21] mitosis, ciliogenesis, hydrolytic enzyme activity, collagen synthesis, and vascular proliferation become prominent during the midproliferative phase of the menstrual cycle.[18, 21] The logarithmic increase and development of estrogen-dependant cellular specializations with progression in the cycle is consistent with a gradually increased estrogenic stimulation upon the endometrial mucosa and coincides with the progressive increase in the levels of circulating preovulatory estradiol-17β. Similarly, the lack of estrogen-dependant alterations in the endometrium during the regenerative period (cycle days 3 and 4) suggests that the cellular events involved in endometrial reconstruction are directed to the replacement and formation of the tissue lost by menstruation and are not influenced by cyclic hormonal stimuli. The hallmark of this series of events is the ability of the residual basal glandular and lower uterine-cornual surface epithelium to form a new surface epithelial membrane.

In view of the great regenerative potential of denuded endometrium in the absence of mitotic activity, analyses using Feulgen microspectrophotometry and quantitative historadioautography with ^{3}H-thymidine are soon to be initiated in this laboratory. These techniques will provide data regarding the content, rate, and localization of nucleic acid synthesis and epithelial renewal time of the resurfacing epithelium, basal glands, and stromal cells. It is our opinion that such studies will contribute to a better understanding of the cytodynamics involved in cyclic endometrial regeneration.

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The barrel-shaped cervical carcinoma

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Twelve patients had barrel-shaped cervical carcinoma among 493 new patients with primary invasive cervical carcinoma, at the Downstate Medical Center, New York, from January, 1964, to December, 1972. The incidence of barrel-shaped lesions among invasive carcinoma of the cervix was 2.43 per cent. All 12 patients were treated with external radiation to the whole pelvis followed by two radium applications. In six patients an extrafascial hysterectomy was performed 6 to 12 weeks after radiation. Six patients died and six survived (50 per cent). Six patients survived for over 2 years after therapy with no evidence of recurrence. Of these, four are alive and well for over 5 years. All six patients died within 20 months after therapy. The barrel-shaped cervical carcinoma fared poorly (six deaths among 12 patients or 50 per cent) when compared with the over-all Stage I and Stage II cervical carcinoma (87 deaths among 339 patients or 25.5 per cent).

THERE IS CONSIDERABLE variation in the gross appearance of cervical carcinoma depending on whether it assumes the exophytic, the endophytic, or ulcerative form. In the endophytic variety the chief direction of growth is into the cervical tissues, and the cervix, therefore, becomes large, nodular, and often stony hard in consistency.[9] Cervical carcinoma characteristically arises from the squamocolumnar junction at the margin of the external os. However, it may occasionally develop entirely within the endocervical canal. The lesion is then apt to be overlooked on speculum examination as the portio vaginalis of the cervix may appear almost normal, though in most instances the intracervical growth soon peeps out from within the canal and becomes visible (Fig. 1). Although Novak[9] did not use the term "barrel-shaped" to describe the lesion, his was probably the earliest description of this variety of cervical carcinoma.

Jeffecoate[7] stated that the diagnosis of cervical carcinoma may be difficult on inspection if the growth is wholly intracervical. The external os looks normal but the cervix as a whole feels big, broad, and barrel-shaped (Fig. 2). This is probably the first time the word "barrel-shaped" was used in the literature to describe this particular type of cervical carcinoma.

However, it was Fletcher and his colleagues[4] at the M.D. Anderson Hospital and Tumor Institute who first considered the barrel-shaped lesion as a special clinical and therapeutic problem. They had observed that some Stage I and Stage II squamous-cell carcinomas of the cervix result more often in treatment failure when the primary lesion grows predominantly within the endocervix. Fletcher and associates[6] described a clinical variety of carcinoma, originating apparently in the endocervix, which remains local and enlarges by expanding the lower corpus until the top and bottom of the uterus has a barrel shape. This special growth pattern produces an especially large local mass within the cervix which needs special attention. These tumors may extend from one side of the pelvis to the other, at times rendering difficult a proper assessment of the parametrial situation. The term "barrel-shaped" is used to distinguish these lesions clinically from the the usual types of carcinoma of the cervix.

The total bulk of tumor in the barrel-shaped cervical carcinoma is greater than implied by the applicable international stage.[4]

Materials and methods

From January, 1964, to December, 1972, 12 patients were treated for barrel-shape type of cervical carcinoma at the Downstate Medical Center. After com-

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plete tumor survey, including blood counts, blood chemistries, urinalysis, chest x-ray, bone survey, intravenous pyelogram, barium enema, cystoscopy, sigmoidoscopy, and any other tests that were clinically indicated, all patients were presented to the Gynecologic Tumor Board. Seven patients were classified as Stage I and five as Stage II.

All 12 patients received external cobalt 3,000 to 4,000 rads to the pelvis over 3 to 4 weeks. This was followed by two radium applications (using intrauterine tandems and vaginal ovoids) 7 to 14 days apart, totaling 5,060 to 8,000 mg. hours. In three patients, an additional 1,000 rads was given to the parametrium during the interval between the first and second radium applications.

An extrafascial hysterectomy is planned 6 weeks after completion of radiation therapy. However, on analyzing our 12 cases of barrel-shaped cervical carcinoma, only 6 patients actually had hysterectomies done.

Six patients were treated by radiation alone. The reasons why hysterectomy was not performed in each case are as follows: In three patients laparotomy revealed nonresectable metastatic tumor; in one patient the Tumor Board did not recommend surgery; two patients refused surgery.

Results

Incidence. At the Downstate Medical Center, for the nine year period, January 1, 1964, to December 31, 1972' there were 12 patients with cervical carcinomas that were classified as barrel shaped. During the same period of time, there were 1,216 new patients with cervical carcinoma. Of these, 671 patients had carcinoma in situ and 645 patients had invasive carcinoma of the cervix. However, 152 had been treated at another institution prior to admission and 493 patients were admitted without prior therapy for their invasive carcinoma of the cervix.

The incidence of barrel-shaped lesions among invasive carcinoma of the cervix is 12 in 493 or 2.43 per cent at this institution.

Clinical data. The youngest patient was 23 years of age and the oldest was 84, with a mean age of 42 years.

The number of pregnancies each patient had ranged from 0 to 10, with a mean of 3.75.

Results of treatment. In this series of 12 patients, six patients died (50 per cent) and six are still alive (50 per cent). All six patients survived for over 2 years after therapy with no evidence of recurrence. Of these, four patients are alive and well after 5 years. The longest survival time is 9 years and 8 months.



Fig. 1. Barrel-shaped cervical carcinoma mainly involving the endocervix. Note the large bulk of the cervix and the apparantly normal smooth appearance of the portio vaginalis.

Various studies have shown that most recurrences and deaths from cervical carcinoma appear within 24 months.[10, 13] In this series all six patients died within 20 months after therapy. The latest death occurred 20 months after therapy and the earliest occurred after 2 months. The average interval of time between therapy and death was 11 months.

Pathology. All 12 patients had invasive epidermoid carcinoma on histologic examination of cervical biopsy prior to therapy. There were no patients with adenocarcinoma.

There were four patients with poorly differentiated epidermoid carcinoma and eight patients with well-differentiated epidermoid carcinoma. All four patients with poorly differentiated epidermoid carcinoma died within 2 years after therapy.

Six patients had hysterectomies performed after radiation therapy. Of these, four are alive and two

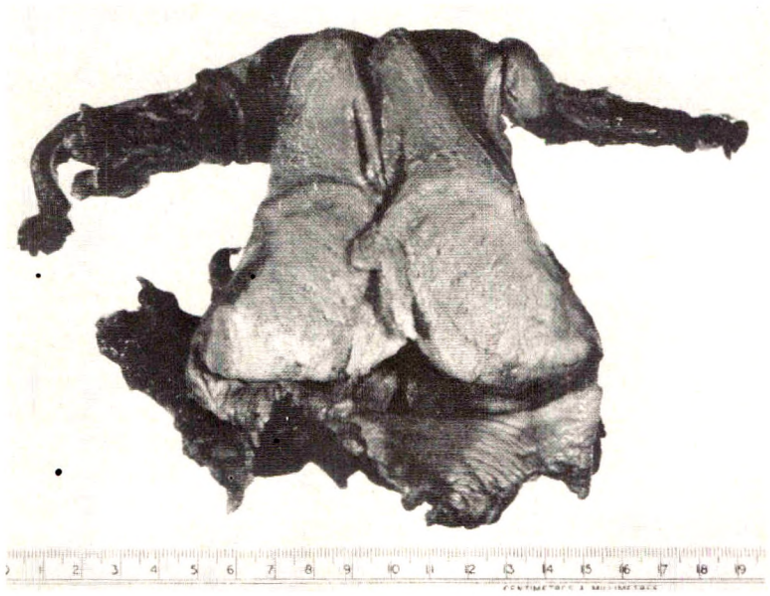


Fig. 2. Carcinoma of the endocervix. Note the barrel-shaped deformity of the cervical canal. (From Howkins, J., and Bourne, G., editors: Shaw's Textbook of Gynecology, ed. 9, London, 1971, Churchill-Livingstone.)

died. Five of the six hysterectomy specimens showed no residual carcinoma in the cervix or uterus, and one had a focus of residual carcinoma in the cervix (16.6 per cent).

Pathology and deaths. Of the six patients who died, four had poorly differentiated epidermoid carcinoma and two had well-differentiated epidermoid carcinoma.

Persistent, recurrent, or metastatic carcinoma was present in all six patients who died.

Two patients had tumor in the pelvis locally, but no evidence of distant metastases. One patient had tumor invading the bladder wall as well as metastases to the para-aortic lymph nodes. Two patients had no evidence of tumor in the pelvis, but had distant metastases to the para-aortic nodes and lumbar vertebrae. One patient had no tumor in the pelvis but had metastases to liver, mesentric lymph nodes, small and large bowels.

Treatment and deaths. Six patients were treated by radiation therapy alone. Of these, four died and two survived (66.6 per cent). Six were treated by preoperative radiation followed by extrafascial hysterectomy. Of these, two died and four survived (33.3 per cent).

Of the six patients who died, each had a complete course of radiation therapy. Only two patients had an extrafascial hysterectomy performed 6 to 12 weeks after radiation.

Treatment and Survivals. Of the six patients who survived, each had a complete course of radiation therapy. In four patients an extrafascial hysterectomy was performed 6 to 12 weeks after radiation. One patient refused surgery after radiation therapy. The other patient was 84 years old and refused surgery.

Comment

The total bulk of cancer in the barrel-shaped cervical carcinoma is greater than implied by the applicable international stage.[4]

Kottmeier,[8] Sherman,[12] Scott and associates,[11] and Bourne have been reluctant to accept the concept of "radioresistance" for local recurrence. They suggested that local failure is related to other factors, including inadequate treatment due to poor geometry.

Radium distribution from intrauterine and vaginal ovoids is not well suited to treat the expanded disease in the lower part of the uterus in patients with bulky endocervical lesions (Fig. 3). Cancer outside the isodose line will be inadequately irradiated. The addition of an extrafascial hysterectomy removes this inadequately treated disease area.[3]

Fletcher[5] and Rutledge at the M.D. Anderson Hospital gave patients with bulky lesions 4,000 rads of whole-pelvis radiation and 4,000 to 5,000 mg. hours of radium. This gives 10,000 rads to the vaginal mucosa and 5,000 rads to the parametrium, obturator, and

external iliac nodes, which is effective for small aggregates of cancer cells in these areas.

An extrafascial hysterectomy removes the disease in the myometrium, which despite some shrinkage after 4,000 rads to the whole pelvis is still at too great a distance from the radium source to receive an effective dose.[5]

Crawford and associates[2] reported from the Confederate Memorial Medical Center, Louisiana, where patients with carcinoma of the cervix Stage I and Stage II were treated by external radiation and radium applications followed in 6 weeks' time by radical hysterectomy in all patients 60 years old and under. Of 137 patients who had surgical procedure, cancerous tissue was removed from 27 (20 per cent) at the time of operation.

In the present series, of the six patients who had hysterectomy done after radiation therapy, five had no residual disease in the uterus or cervix and one had a focus of invasive carcinoma in the cervix (16.6 per cent).

Suit and associates[13] found 226 recurrences in a total of 1,190 patients with cervical carcinoma treated by radiotherapy at the M.D. Anderson Hospital. Of these, only 22 appeared after the twenty-fourth month following treatment. They concluded that recurrence occurs early, predominantly within the first 24 months after treatment.

In this series of 12 patients with barrel-shaped cervical carcinoma treated at the Downstate Medical Center between 1964 and 1972, six patients died (50 per cent) and six survived (50 per cent). All six patients died within 20 months after therapy. The mean interval between therapy and death was 11 months. The remaining six patients had survived for over 2 years after therapy with no evidence of recurrence. Of these, three are alive and well after 5 years.

For the same 9 year period, 1964 and 1972, there were 345 new patients with Stage I and Stage II carcinoma of the cervix treated at the Downstate Medical Center. Seven patients were lost to follow-up. Of the remaining 339 patients, 87 died (25.5 per cent) and 251 survived (74.5 per cent). Of these deaths, 65 died within 2 years after therapy and 22 died after more than 2 years.

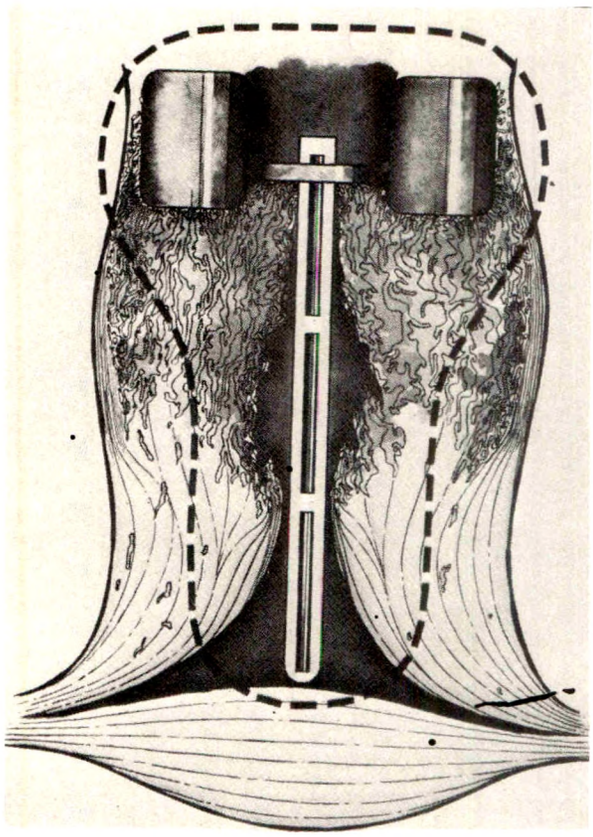


Fig. 3. Bulky expansile lesion of endocervix with extension to myometrium. Pear-shaped broken line corresponds to 7,000 rads isodose curve. Cancer outside the broken line will be inadequately irradiated but is well located for surgical removal by hysterectomy. (From Fletcher, G. H.: Textbook of Radiotherapy, ed. 2, Philadelphia, 1973, Lea & Febiger, Publishers.)

It is immediately apparent that the barrel-shaped cervical carcinoma fared badly when compared with the rest of our Stage I and Stage II carcinoma of the cervix.

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Comparison of culdoscopic and laparoscopic tubal sterilization

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The growing number of women requesting interval sterilization (apart from term delivery or abortion procedures) necessitates the re-evaluation of traditional surgical procedures. A total of 800 women—the first 400 women undergoing culdoscopic and laparoscopic tubal sterilization procedures at Siriraj Hospital in Bangkok, Thailand—evaluate the appropriateness of the two sterilization procedures for use in interval sterilization programs. No serious operative complications occurred in either group. However, the rate of postoperative complications was considerably higher for the culdoscopic group than for the laparoscopic group. Hospitalization was required for only a small percentage of the patients and most had resumed their normal activities within two days of the operation. This study indicates that both procedures are effective and may be safely performed on an outpatient basis without general anesthetics and with minimal discomfort for the patients.

IN AN AGRICULTURAL country like Thailand where the majority of people get married while still quite young and do not concern themselves with methods of contraception until they have achieved their desired family size, a permanent contraceptive method seems more appropriate than a temporary method. Postpartum tubal sterilization has been practiced in almost all Thai hospitals for over 30 years. In Siriraj Hospital, the proportion of cases sterilized after term delivery has varied from 8 to 17 per cent over the past 20 years.

Although postpartum sterilization remains the most popular method, there is a rapidly increasing demand for interval sterilization. However, using the traditional surgical methods of sterilization for interval cases is unsatisfactory and many new techniques are being developed for use with these patients. The development and testing of these methods should be a priority in family-planning programs. A desirable method should meet the following criteria: (1) the method should be highly effective and involve only minimal risk to the patient; (2) it should be readily available as an outpatient procedure; (3) it should require only simple and inexpensive equipment; (4) it should cause only minimal discomfort to the patient without the use of general anesthetic; and (5) it should leave no noticeable scars and not interfere with the rapid resumption of normal activities.

Culdoscopic and laparoscopic methods of sterilization are among several approaches currently under investigation which meet many of these criteria. The purpose of this study is to evaluate culdoscopic and laparoscopic methods of sterilization for use in developing countries.

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Materials and methods

The study group consists of 800 women—the first 400 women undergoing culdoscopic and laparoscopic tubal sterilization procedures at Siriraj Hospital in Bangkok, Thailand. Both methods were performed by the same group of three surgeons or by second year residents assisted by the same team for training purposes. The culdoscopic method was introduced at Siriraj Hospital in September, 1971. Later, in April 1972, when both methods were available, the procedures were randomly assigned to patients, except in

Table I. Pre-existing medical conditions

Pre-existing condition	Culdoscopy		Laparoscopy		Total	
	No.	%	No.	%	No.	%
None	385	96.3	374	93.5	759	94.9
Prior pelvic/ abdominal surgery	4*	1.0	8†	2.0	12	1.5
Systemic disorder	11†	2.7	13§	3.2	24	3.0
Other	—	—	5‖	1.3	5	0.6
Total	400	100.0	400	100.0	800	100.0

*Postpartum sterilization (failed) 1, previous cesarean section 1, appendectomy 1, repair of umbilical hernia 1.

†Thalassemia 3, hypertension 2, pulmonary tuberculosis 2, bronchial asthma 2, hypothyroidism 1, systemic lupus erythematosus 1.

‡Salpingo-oophorectomy (ovarian tumor) 1, failed culdoscopy 1, septic (criminal) abortion 1, appendectomy 2, cervical conization 2, extrauterine Cu T 1.

§Thalassemia 2, diabetes mellitus 2, pulmonary tuberculosis 2, thyrotoxicosis 2, hypertension 3, nephrotic syndrome 1, ascariasis 1.

‖Mental retardation 2, fracture thoracic spine (deformity) 1, umbilical hernia 1, left pneumonectomy 1.

cases where there were contraindications to one of the methods.

A complete medical history was obtained and physical, vaginal, and laboratory examination, including a chest x-ray, were performed. Culdoscopy was not performed in cases where there was a history of pelvic inflammatory disease or vaginal infection. A free cul-de-sac is mandatory for this procedure. Very obese patients were also excluded because of the need to maintain the knee-chest position during this procedure. Laparoscopy was not performed in women with extensive abdominal scars, heart disease, or serious pulmonary diseases. Women with a previous history of pelvic infection, however, were not excluded, provided that the infection had subsided. Over 90 per cent of the patients in both groups had no pre-existing medical conditions (Table I).

Ovarian biopsies were performed concurrently with 48 culdoscopic sterilizations to study the effects of hormonal contraceptives. Anterior and/or posterior colporraphy was performed on 12 cases of culdoscopic and 3 cases of laparoscopic sterilization. One extrauterine copper T IUD (Cu T) was removed via laparoscope (the details have been separately reported[1]).

The patients were usually scheduled for operation during the first ten days of their menstrual cycles after cessation of menstruation. However, if the patient had been using a reliable contraceptive method, the operation was performed at any time during the menstrual cycle.

Table II. Per cent distribution of laparoscopic and culdoscopic tubal sterilization cases by selected characteristics

Characteristics	Culdoscopy (N = 400)	Laparoscopy (N = 400)	Total
Residence:			
Bangkok	54.7	54.2	54.5
Other	45.3	45.8	45.5
Age:			
<24	7.0	7.8	7.4
25-29	26.8	27.5	27.1
30-34	39.5	35.7	37.6
35-39	22.7	24.3	23.5
>40	4.0	4.7	4.4
Education:			
None	5.5	8.0	6.7
Primary	77.3	77.5	77.4
Secondary	10.0	9.2	9.6
Vocational	5.7	4.0	4.9
University	1.5	1.3	1.4
Religion:			
Buddhist	97.0	96.5	96.7
Confucian	1.7	2.0	1.9
Catholic	0.8	1.3	1.0
Muslim	0.5	0.2	0.4
Occupation:			
Housewife	43.3	50.8	47.0
Farmer	17.7	21.0	19.4
Professional or skilled worker	14.0	12.0	13.0
Sales worker	14.3	9.7	12.0
Unskilled worker	10.7	6.5	8.6
Income (both):			
<999	35.3	42.3	38.7
1,000-1,499	29.7	27.5	28.6
1,500-1,999	9.5	9.2	9.4
>2,000	25.5	21.0	23.3
Living children:			
0-1	0.8	1.0	0.9
2	2.0	2.3	2.1
3	36.2	39.7	38.0
4	28.3	27.7	28.0
>5	32.7	29.3	31.0

The two patient groups were similar in age, parity, residence, education, and employment status (Table II). All but 15 cases requested sterilization as a means of limiting family size. A majority of the women had three or more living children and approximately 30 per cent of both groups had five or more living children. Table III gives a breakdown of those sterilized for medical or eugenic reasons.

Procedures. All patients were instructed to give themselves rectal enemas the morning of the day of the operation and to take nothing by mouth before reporting to the hospital at 7:30 A.M. on the day surgery was scheduled. Before entering the operating room patients were requested to empty their bladders.

In culdoscopic procedures intravenous pethedine (100 mg.) was administered after the patient had assumed the knee-chest position. The use of chlorpromazine in combination with pethidine was tried in a few cases, but was discontinued as unnecessary and potentially dangerous. Without a special operating table, general anesthetics could not be used in culdoscopic procedures.

For patients undergoing laparoscopic procedures, different combinations of intravenously administered neuroleptanalgesics were tried: (1) pethidine (100 mg.) in combination with 10 mg. of diazepam (Valium); (2) pethidine (100 mg.) in combination with 2.5 mg. of droperidol; (3) fentanyl (0.05 mg.) in combination with 5 mg. of droperidol; and (4) fentanyl (0.05 mg.) in combination with 10 mg. of diazepam. The dosage was slightly decreased or increased in markedly under or overweight patients. General anesthetic was used in only two cases: one patient with Down's syndrome and one patient for whom an extrauterine Cu T was concurrently removed. Xylocaine (1 per cent) was used for local infiltration in both procedures.

Culdoscopic procedures employed a modified Pomeroy's technique as described by Clyman[2] and demonstrated to the authors by Alfonso Gutierrez-Najar. Laparoscopic procedures employed electric cauterization of the uterine tubes via a single incision laparoscopy as described by Clifford R. Wheeless,[3] with slight modification. A combined sound-tenaculum* was used to move the uterus instead of the Rubin cannula. In the first 100 laparoscopic cases, the burned part of the uterine tube was excised, but for the latter 300 cases, both cut ends of the tubes were recauterized. Occasional exceptions to these procedures will be discussed later.

If only neuroleptanalgesics or local anesthetics were used, the patients got up immediately after the operation and walked to the recovery room beside the operating room. There, they were observed for 2 to 3 hours, after which they were discharged in the company of husbands or relatives. Routine prophylactic antibiotics were given for 5 days after the operation.

Patients were scheduled for follow-up visits at 1 week, 6 months, and 1 year after the operation and yearly thereafter. Cases living in distant provinces were referred to local health centers which were asked to send the follow-up results to the Siriraj Hospital Unit. The longest and shortest periods of follow-up reported in these series were 40 months and 3 months, respectively, with a total of 11,084 months' observation.

*Designed by Jaroslav F. Hulka, M.D., of the University of North Carolina at Chapel Hill.[4]

Table III. Reason for sterilization

Reason for sterilization	*Culdoscopy*		*Laparoscopy*	
	No.	*%*	*No.*	*%*
Family size limitation	394	98.5	391	97.7
Medical or eugenic reason				
Thalassemia	3	0.7	2	0.5
S.L.E.	1	0.3	—	—
Hypothyroidism	1	0.3	—	—
Carcinoma of nasopharynx	1	0.3	—	—
Thyrotoxicosis	—	—	2	0.5
Diabetes mellitus	—	—	2	0.5
Nephrotic syndrome	—	—	1	0.3
Down's syndrome	—	—	2	0.5
Total	400	100.0	400	100.0

Results

Anesthesia complications. As previously stated, local anesthetic and intravenous pethidine were administered before the operation. All culdoscopic patients remained fully conscious during the operation and the majority felt only slight or bearable pain. Only a few patients became restless or felt severe pain during the operation (Table IV). In laparoscopic cases, different combinations of neuroleptanalgesics were used. Pethidine and droperidol seemed to be the better of the two most frequently used combinations. The majority of the patients so treated were still conscious and cooperative, but felt slight or bearable pain (Table IV). Because of the small number of cases using other combinations, analysis was not possible.

Technical difficulties. Pelvic adhesions resulting from previous infection or surgery were the most common cause of operative difficulties in both procedures. Although women with a history of pelvic inflammatory disease were excluded from the group undergoing culdoscopy, moderate pelvic adhesions and/or hydrosalpinx were found in nine cases (2.2 per cent). In two cases, the tubes could not be pulled through the vaginal incision and tubal sterilization was impossible. In the laparoscopic group, where suspected pelvic adhesions were not a contraindication for the procedure, adhesions were encountered in 8.7 per cent of the patients. In all but six of the cases where cauterization could not be properly done because of thick adhesions, the area close to the cornu of the uterus was burned. Salpingograms performed 3 months later showed complete obstruction of both tubes in these cases. Because of very thick pelvic adhesions, cauterization of both tubes was impossible in two cases.

In the culdoscopic group, additional operative

Table IV. Comparison of neuroleptanalgesics used in laparoscopic cases

Type of neuroleptanalgesic	*No. of cases*	*Effect*					
		Almost asleep		*Conscious and cooperative*		*Restless*	
		No.	*%*	*No.*	*%*	*No.*	*%*
Pethidine (100 mg.) and Valium (10 mg.)	237	153	64.6	68	28.7	16	6.7
Pethidine (100 mg.) and droperidol (25 mg.)*	120	18	15.0	101	84.2	26	66.7
Fentanyl (0.01 mg.)† and droperidol (2.5 mg.)	39	16	6.7	1	0.8	2	5.1

*Five patients with moderate pelvic adhesions experienced severe pain.
†Three patients with moderate pelvic adhesions experienced severe pain.

Table V. Operative complications for culdoscopic and laparoscopic procedures

Operative complications	*Culdoscopy*		*Laparoscopy*	
	No.	*%*	*No.*	*%*
Separation of 2 layers of cul-de-sac	3	0.7	—	—
Tear of uterine tube or broad ligament	2	0.5	—	—
			—	—
Hematoma of broad ligament	1	0.2	—	—
Subcutaneous emphysema	—	—	3	0.7
Blood oozing requiring recauterizing	—	—	7	1.7

Table VI. Postoperative complications

Postoperative complications	*Culdoscopy (N = 400)*		*Laparoscopy (N = 400)*	
	No.	*%*	*No.*	*%*
Shoulder pain	156	39.0	17	4.2
Infected skin incision	—	—	17	4.2
Polyp-like granulation	15	3.7	—	—
PID and/or abscess	13*	3.2	—	—
Dyspareunia	10	2.5	—	—

*Three of these (0.7 per cent) required laparotomy.

difficulties occurred as a result of the patients' inability to maintain the knee-chest position. In one case in which the patient was unable to maintain the knee-chest position, only one uterine tube was occluded.

The over-all technical failure rates were 0.7 per cent and 0.5 per cent in the culdoscopic and laparoscopic groups, respectively.

Operative complications. Separation of the two layers of the cul-de-sac occurred in 0.7 per cent of the culdoscopic cases, while among laparoscopic cases subcutaneous or subfascial emphysema of the abdominal wall occurred in 0.7 per cent of the cases (Table V).

Tearing of the uterine tubes and broad ligament occurred in two culdoscopic cases (0.5 per cent) when the tubes were pulled out, resulting in slightly more bleeding than usual. This might be compared to bleeding from the cut surface of the tubes after cauterization and excision in laparoscopic cases (1.7 per cent). All cases were easily managed, however, and no serious complications ensued. A small hematoma of the broad ligament occurred in one culdoscopic case.

Operative and hospitalization times. The operative time was calculated from the first puncture to closure. The mean surgical time was 12.0 minutes for culdoscopy and 10.6 minutes for laparoscopy with a range of 5 to 30 minutes. Training and demonstration cases are included in this estimate. The majority of patients left the hospital 2 to 3 hours after the operation and resumed their usual household duties by the next day. Only patients with concurrent surgery were admitted to the hospital.

Postoperative complications. Complication rates were significantly higher for patients undergoing culdoscopy than for patients having laparoscopy (Table VI). Significant pelvic infection was found in 13 culdoscopic cases, of which 8 patients had a definite pelvic mass. None of these patients showed significant signs and symptoms until about 10 to 20 days after the operation. Satisfactory results were obtained with conservative treatment in 10 cases. The other three patients developed pelvic abscess which could not be controlled conservatively and required abdominal hysterectomy and removal of tuboovarian abscess at 12, 20, and 39 days after tubal sterilization. Because vaginal examination was not routinely done in culdoscopic cases at the seventh postoperative day, incidence of infected cul-de-sac wound can not be reported.

However, there were no clinical signs and symptoms observed. Polyp-like granulation tissue was found on examination of the cul-de-sac incision 1 month after the operation in 3.7 per cent of the cases. This polyp-like granulation was effectively treated by electric or chemical cauterization.

No significant pelvic infection was found following surgery in the laparoscopic group despite the fact that 35 of these patients were known to have pre-existing pelvic inflammatory disease. Infected abdominal incisions were reported in 4.2 per cent of the group.

At the one-month follow-up visit, 2.5 per cent of the culdoscopic patients reported dyspareunia, without any sign of pelvic or vaginal infection. However, this symptom disappeared a few months later. Dyspareunia was not reported in laparoscopic cases.

While 39 per cent of the patients having culdoscopic procedures reported shoulder pain lasting for a few days after the operation, only 4.2 per cent of the patients having laparoscopic procedures complained of shoulder pain. The difference in frequency of this complaint is due to the slow absorption of air in culdoscopic cases and the rapid absorption of the carbon dioxide used in laparoscopic cases. This complication could be minimized by removing as much air as possible before closing the incision.

Pregnancy rate. One pregnancy occurred in the culdoscopic group: a 32-year-old woman became pregnant 23 months after the procedure. She was 3 months pregnant at the cut-off date of this report. No pregnancies occurred in the laparoscopic group subsequent to the operation. However, two patients, who had given incorrect information on their menstrual cycles because they were afraid they would be denied the operation, were operated on during the second half of the menstrual cycle and were later found to be pregnant. The operation had no effect on either pregnancy. One patient delivered a term normal infant and the other woman was still pregnant at the close of the study.

Comment

The two patient groups (400 undergoing culdoscopy and 400 undergoing laparoscopy) were similar in respect to residence, age, education, religion, occupation, and number of living children. Nearly all of the patients had requested sterilization as a means of limiting family size. Most of the patients (96.3 per cent, culdoscopy group and 93.5 per cent, laparoscopy group) had no pre-existing medical conditions. There were a higher number of patients with pre-existing conditions in the laparoscopic group because while previous pelvic inflammatory disease was considered a contraindication for culdoscopy, it was not for laparoscopy. Concurrent operative procedures (ovarian biopsy, anteroposterior or posterior repair, IUD removal) were performed in 60 culdoscopy cases and in 4 laparoscopy cases.

While nearly all of the patients having culdoscopic procedures were administered intravenous pethidine (100 mg.), patients having laparoscopic procedures were administered several combinations of neuroleptanalgesics. The most effective combination seemed to be pethidine (100 mg.) with 2.5 mg. of droperidol. The majority of the patients so treated (84. 2 per cent) were conscious and cooperative, but felt slight (61.7 per cent) or bearable pain (23.3 per cent). Patients having culdoscopic procedures were less heavily sedated because of the necessity that these patients be able to maintain the knee-chest position.

The majority of the technical difficulties encountered in both procedures were caused by the presence of pelvic adhesions. In two culdoscopic cases (of the 9 cases discovered to have pelvic adhesions), the procedure could not be performed. Since a history of pelvic inflammatory disease was not a contraindication for laparoscopy, more of these patients had pelvic adhesions (35 cases). The procedure could not be properly performed in 4 cases and was impossible in two other cases. In one culdoscopic case the procedure was not completed because of the patient's inability to maintain the knee-chest position.

Operative complications were similar for both groups. No serious operative complications were encountered in either group. However, the rate of postoperative complications was significantly higher for the culdoscopic group. While only 8.4 per cent of the patients having laparoscopy experienced minor complications (shoulder pain and infected skin incisions), 13 of the patients undergoing culdoscopy were treated for pelvic infection or abscess. Nearly half of the patients having a culdoscopy experienced one or more complications; however, most of these complications were minor (39.0 per cent, shoulder pain; 3.7 per cent, polyp-like granulation; 2.5 per cent, dyspareunia). No deaths occurred in either group.

This study indicates that both procedures are effective and may be safely performed on an outpatient basis. Both may be of particular value in developing countries where hospital facilities and physician time are in short supply.

We wish to express our sincere thanks to Prof. Prabhand Ariyamitr, Drs. Alfonso Gutierrez-Najar,

Clifford R. Wheeles, Jerry F. Hulka, Elton Kessel, Nicholas Wright, and Ms. Linda W. Lewis for their help, and to all personnel in the Family Planning Research Unit, Siriraj, who assisted the authors with this study.

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Some straight talk about acute cystitis therapy.

There's been a lot of discussion about what constitutes the best therapy for acute cystitis. Issues are being raised about resistance, fecal flora, spectrum of activity. But the important issues are still efficacy, safety and economy. Gantrisin® (sulfisoxazole) offers a superior balance of these three basic needs in acute cystitis.[1-4]* Now, a new study confirms Gantrisin efficacy. In this study, as often occurs in medical practice, therapy was initiated at the same time cultures were taken. The results of this study corroborate one fact. When you suspect gram-negative organisms† while awaiting culture results, you can prescribe Gantrisin with confidence. The results? 89% of 251 patients studied achieved sterile urine. A straight 89%.



*nonobstructed; due to susceptible organisms.

†Susceptible strains of *E. coli, Klebsiella-Aerobacter, P. vulgaris, P. mirabilis* and also gram-positive *Staph. aureus.*

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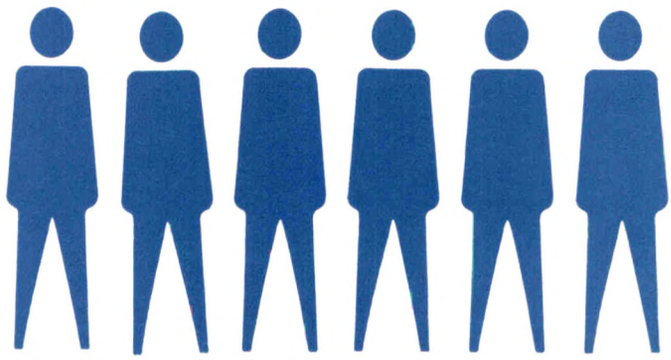
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Straight talk about Gantrisin® in acute cystitis*

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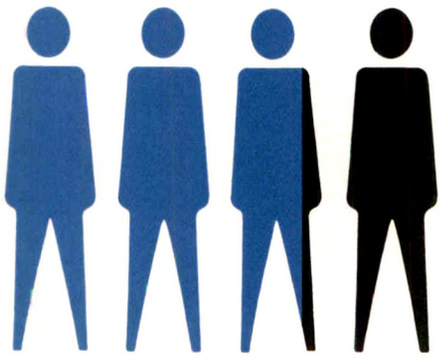
In a new study of Gantrisin (sulfisoxazole) in acute lower urinary tract infections, 89% of patients achieved sterile urine.†



No. of patients	Sterile urine	Clear culture (1000 or less organisms/ml urine)	Clear culture (10,000 or less organisms/ml urine)
251	89% (224)	90% (226)	92% (232)

Over the years Gantrisin (sulfisoxazole) has been the subject of continuing clinical research. There are many references which attest to its high degree of efficacy in acute cystitis. Now a new clinical study of acute lower urinary tract infection again demonstrates Gantrisin effectiveness. The pathogens causing these infections were *E. coli, Klebsiella-Aerobacter, P. vulgaris* and *P. mirabilis,* and the results—Gantrisin achieved 89% effectiveness.

*nonobstructed; due to susceptible organisms
†Data on file, Hoffmann-La Roche Inc., Nutley, New Jersey 07110



Gantrisin (sulfisoxazole) offers an established record of safety

Gantrisin introduced a new era of sulfonamide therapy coupling effectiveness and relative safety.[1-3] Side effects are few. In this study only 2% of the patients experienced side effects, which were mild. These included drowsiness, fatigue, rash, pruritus, diarrhea, nausea, vomiting, headache. The high solubility of Gantrisin minimizes risk of crystalluria and renal damage. However, as with any sulfonamide therapy, maintain adequate fluid intake and perform frequent urinalyses with careful microscopic examination.

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Gantrisin (sulfisoxazole) costs less than most therapies

Few antibacterials can approach the economy of Gantrisin therapy. The average cost of 10-day Gantrisin therapy is less than 10 days of most other antibacterials.

Before prescribing, please consult complete product information, a summary of which follows:

Indications: Nonobstructed urinary tract infections (mainly cystitis, pyelitis, pyelonephritis) due to susceptible organisms.

IMPORTANT NOTE: *In vitro* sensitivity tests not always reliable; must be coordinated with bacteriological and clinical response. Add aminobenzoic acid to follow-up culture media. Increasing frequency of resistant organisms limits usefulness of antibacterial agents, especially in chronic and recurrent urinary infections. Maximum safe total sulfonamide blood levels, 20 mg/100 ml; measure levels as variations may occur.

Contraindications: Hypersensitivity to sulfonamides; infants less than 2 months of age; pregnancy at term and during the nursing period.

Warnings: Safety in pregnancy not established. Do not use for Group A beta-hemolytic streptococcal infections, as sequelae (rheumatic fever, glomerulonephritis) are not prevented. Deaths reported from hypersensitivity reactions, agranulocytosis, aplastic anemia and other blood dyscrasias. Sore throat, fever, pallor, purpura or jaundice may be early indications of serious blood disorders. CBC and urinalysis with careful microscopic examination should be performed frequently.

Precautions: Use cautiously in patients with impaired renal or hepatic function, severe allergy or bronchial asthma. Hemolysis, frequently dose-related, may occur in glucose-6-phosphate dehydrogenase-deficient patients. Maintain adequate fluid intake to prevent crystalluria and stone formation.

Adverse Reactions: *Blood dyscrasias:* Agranulocytosis, aplastic anemia, thrombocytopenia, leukopenia, hemolytic anemia, purpura, hypoprothrombinemia and methemoglobinemia; *Allergic reactions:* Erythema multiforme (Stevens-Johnson syndrome), generalized skin eruptions, epidermal necrolysis, urticaria, serum sickness, pruritus, exfoliative dermatitis, anaphylactoid reactions, periorbital edema, conjunctival and scleral injection, photosensitization, arthralgia and allergic myocarditis; *Gastrointestinal reactions:* Nausea, emesis, abdominal pains, hepatitis, diarrhea, anorexia, pancreatitis and stomatitis; *C.N.S. reactions:* Headache, peripheral neuritis, mental depression, convulsions, ataxia, hallucinations, tinnitus, vertigo and insomnia; *Miscellaneous reactions:* Drug fever, chills and toxic nephrosis with oliguria and anuria. Periarteritis nodosa and L.E. phenomenon have occurred. Due to certain chemical similarities with some goitrogens, diuretics (acetazolamide, thiazides) and oral hypoglycemic agents, sulfonamides have caused rare instances of goiter production, diuresis and hypoglycemia as well as thyroid malignancies in rats following long-term administration. Cross-sensitivity with these agents may exist.

Dosage: Contraindicated in infants under 2 months except in the treatment of congenital toxoplasmosis as adjunctive therapy with pyrimethamine.

Usual adult dosage—2 to 4 Gm initially, then 4 to 8 Gm/24 hrs in 4 to 6 doses. *Usual dosage for infants over 2 months and children*—½ 24-hrs dose initially, then 150 mg/kg/24 hrs in 4 to 6 doses; not over 6 Gm/24 hrs.

How Supplied: Tablets containing 0.5 Gm sulfisoxazole, white, scored—bottles of 100, 500 and 1000; drums of 5000; Tel-E-Dose® packages of 100; Prescription Paks of 100, available singly and in trays of 10.

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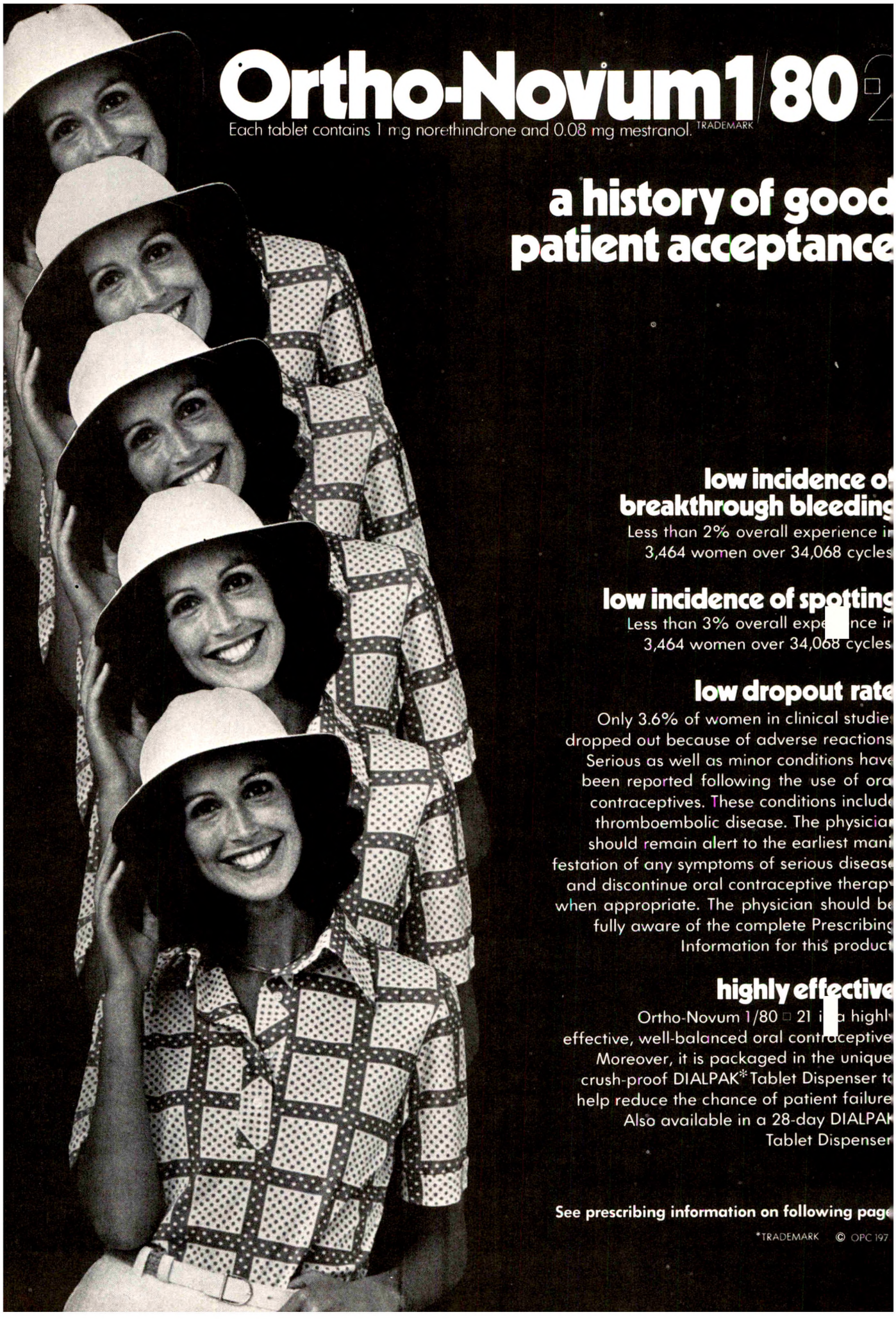
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Gantrisin®

sulfisoxazole/Roche

4 to 8 tablets (0.5 Gm each) *stat*, 2 to 4 tablets *q.i.d.*

A superior balance of efficacy, safety and economy in acute cystitis therapy



Ortho-Novum 1/80 □ 21

Each tablet contains 1 mg norethindrone and 0.08 mg mestranol. TRADEMARK

a history of good patient acceptance

low incidence of breakthrough bleeding

Less than 2% overall experience in 3,464 women over 34,068 cycles

low incidence of spotting

Less than 3% overall experience in 3,464 women over 34,068 cycles

low dropout rate

Only 3.6% of women in clinical studies dropped out because of adverse reactions. Serious as well as minor conditions have been reported following the use of oral contraceptives. These conditions include thromboembolic disease. The physician should remain alert to the earliest manifestation of any symptoms of serious disease and discontinue oral contraceptive therapy when appropriate. The physician should be fully aware of the complete Prescribing Information for this product.

highly effective

Ortho-Novum 1/80 □ 21 is a highly effective, well-balanced oral contraceptive. Moreover, it is packaged in the unique crush-proof DIALPAK* Tablet Dispenser to help reduce the chance of patient failure. Also available in a 28-day DIALPAK Tablet Dispenser.

See prescribing information on following page

*TRADEMARK © OPC 197

Ortho-Novum 1/80□21 and 28

TRADEMARK

Each white tablet contains 1 mg norethindrone and 0.08 mg mestranol.
Each green tablet in the 28-day regimen contains inert ingredients.

a history of good patient acceptance



Ortho-Novum 1/80□21 and Ortho-Novum 1/80□28

Description: Each white ORTHO-NOVUM 1/80 tablet contains 1 mg of the progestational substance, norethindrone, together with 0.08 mg of the estrogenic compound, mestranol. Each green tablet in the 28-day regimen contains inert ingredients.

Actions: ORTHO-NOVUM 1/80□21 and ORTHO-NOVUM 1/80□28 act through the mechanism of gonadotrophin suppression, i.e., through the estrogenic and progestational actions of the ingredients. Alterations in cervical mucus and in the endometrium may also contribute to the efficacy of ORTHO-NOVUM 1/80□21 and ORTHO-NOVUM 1/80□28.

Special note: Oral contraceptives have been marketed in the United States since 1960. Reported pregnancy rates vary from product to product. The effectiveness of the sequential products appears to be somewhat lower than that of the combination products. Both types provide almost completely effective contraception.

The physician should consider a thorough discussion with each patient about the prescribing information, risks and benefits associated with oral contraceptives, taking into account various factors such as age and other physical conditions.

An increased risk of thromboembolic disease associated with the use of hormonal contraceptives has now been shown in studies conducted in both Great Britain and the United States. Other risks, such as those of elevated blood pressure and reduced tolerance to carbohydrates, have been reported and appropriate tests should be conducted to monitor these during oral contraceptive therapy. Liver disease has also been reported, and the physician should be alert to its earliest manifestations.

Long-term administration of both natural and synthetic estrogens in subprimate animal species in multiples of the human dose increases the frequency for some animal carcinomas. These data cannot be transposed directly to man. The possible carcinogenicity due to the estrogens can neither be affirmed nor refuted at this time. Close clinical surveillance of all women taking oral contraceptives must be continued.

Indication: Contraception.

Contraindications: 1. Thrombophlebitis, thromboembolic disorders, cerebral vascular disease, myocardial infarction, or a past history of these conditions. 2. Markedly impaired liver function. 3. Known or suspected carcinoma of the breast. 4. Known or suspected estrogen-dependent neoplasia. 5. Undiagnosed abnormal genital bleeding. 6. Known or suspected pregnancy.

Warnings: 1. The physician should be alert to the earliest manifestations of thrombotic and thromboembolic disorders, thrombophlebitis, cerebrovascular disorders including hemorrhage, pulmonary embolism and retinal thrombosis. Should any of these occur or be suspected, the drug should be discontinued immediately.

Retrospective studies of morbidity and mortality in Great Britain and studies of morbidity in the United States have shown a statistically significant association between thrombophlebitis, pulmonary embolism and cerebral vascular disease, occlusive or hemorrhagic, and the use of oral contraceptives. There have been three principal studies in Great Britain[1-3] leading to these conclusions and three in this country.[4-7] The estimate of the relative risk of thromboembolism in the study by Vessey and Doll[3] was about sevenfold, while the United States studies found relative risks of 4.4 to 11, meaning that the users are several times as likely to undergo thromboembolic disease without evident cause as nonusers.

In May, 1974, the Royal College of General Practitioners in England[8] issued an interim report of its continuing large-scale prospective study comparing a user group to a nonuser group. This study in its interim analysis states, "A statistically significant higher rate of reporting of cerebrovascular accidents in Takers is evident, but the numbers are too small to justify an estimation of the degree of risk." The study also reported a higher incidence of superficial and deep vein thrombosis in users as compared to nonusers. The risk of superficial and deep vein thrombosis was reported to be lower in women using 50 mcg estrogen preparations.

The Sartwell study[4] indicated that the risk did not persist after discontinuation of administration. Both the Sartwell and the Royal College studies indicated that the degree of risk was not associated with duration of treatment.

In a collaborative American study[5,6] of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the relative risk of thrombotic stroke was 4.1 to 9.5 times greater in users than in nonusers. A comparable estimate for hemorrhagic stroke was 2.0.

None of the American studies was designed to evaluate a difference between products. However, the Sartwell study[4] suggested that there might be an increased risk of thromboembolic disease in users of sequential products.

Other retrospective studies[9,10] have reported an increased risk of post-surgery thromboembolic complications in oral contraceptive users. It has been recommended that therapy be discontinued at least one month prior to elective surgery.

In May of 1975 two separate retrospective case control studies relating to fatal and nonfatal myocardial infarction in users and nonusers of oral contraceptives were reported.[11,12] The studies estimate that the increased risk of hospital admission for myocardial infarction associated with the use of oral contraceptives as compared to nonusers is 2.7 times in the 30-39 age group and 5.7 times in the 40-44 age group; and the risk of death associated with the use of oral contraceptives as compared to nonusers was increased 2.8 times for users in the 30-39 age group and 4.7 times in the 40-44 age group. One of these studies reported that oral contraceptive users who had myocardial infarction were more likely to be heavy smokers and have certain additional risk factors, i.e., treated hypertension and diabetes, pre-eclamptic toxemia, obesity and type II hyperlipoproteinemia.

2. Discontinue oral contraceptive medication if there is: gradual or sudden partial or complete loss of vision; proptosis or diplopia; onset or aggravation of migraine or development of headache of a new pattern which is recurrent, persistent or severe; papilledema; or any evidence of retinal vascular lesions.

3. Fetal abnormalities have been reported to occur in the offspring of women who have taken progestogens and/or estrogens during pregnancy.[13,14] The safety of ORTHO-NOVUM 1/80□21 or ORTHO-NOVUM 1/80□28 in pregnancy has not been demonstrated. Pregnancy should be ruled out before initiating or continuing the contraceptive regimen. Pregnancy should always be considered if withdrawal bleeding does not occur.

4. A small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The long-range effect to the nursing infant cannot be determined at this time.

5. Hepatic lesions (adenomas, hepatomas, hamartomas, regenerating nodules, etc.), occasionally fatal, have been reported in women on oral contraceptives. Such lesions may present as an abdominal mass or with the signs and symptoms of an acute abdomen. These lesions should be considered if the patient has abdominal pain or evidence of intra-abdominal bleeding. This has been reported in short-term as well as long-term users of oral contraceptives.

Precautions: 1. A thorough history and physical examination should be performed before prescribing oral contraceptives and periodically during their administration and should include special reference to breasts and pelvic organs, including Papanicolaou smear since estrogens have been known to produce tumors, some of them malignant, in five species of subprimate animals. 2. Endocrine and possibly liver function tests may be affected by treatment with ORTHO-NOVUM 1/80□21 or ORTHO-NOVUM 1/80□28. Therefore, if such tests are abnormal in a patient taking ORTHO-NOVUM 1/80□21 or ORTHO-NOVUM 1/80□28, it is recommended that they be repeated after the drug has been withdrawn for two months. 3. Under the influence of estrogen-progestogen preparations, pre-existing uterine fibromyomata may increase in size. 4. Because these agents may cause some degree of fluid retention, conditions which might be influenced by this factor, such as epilepsy, migraine, asthma, cardiac or renal dysfunction, require careful observation. 5. In breakthrough bleeding, and in all cases of irregular bleeding per vaginam, nonfunctional causes should be borne in mind. In undiagnosed bleeding per vaginam, adequate diagnostic measures are indicated. 6. Patients with a history of psychic depression should be carefully observed and the drug discontinued if the depression recurs to a serious degree. 7. Any possible influence of prolonged ORTHO-NOVUM 1/80□21 or ORTHO-NOVUM 1/80□28 therapy on pituitary, ovarian, adrenal, hepatic or uterine function awaits further study. 8. A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. The mechanism of this decrease is obscure. For this reason, diabetic patients should be carefully observed while receiving ORTHO-NOVUM 1/80□21 or ORTHO-NOVUM 1/80□28 therapy. 9. The age of the patient constitutes no absolute limiting factor, although treatment with ORTHO-NOVUM 1/80□21 or ORTHO-NOVUM 1/80□28 may mask the onset of the climacteric. 10. The pathologist should be advised of ORTHO-NOVUM 1/80□21 or ORTHO-NOVUM 1/80□28 therapy when relevant specimens are submitted. 11. Susceptible women may experience an increase in blood pressure following administration of contraceptive steroids. 12. Cholestatic jaundice has been reported in users of oral contraceptives. If this occurs ORTHO-NOVUM 1/80□21 or ORTHO-NOVUM 1/80□28 should be discontinued. This condition is more likely to occur in patients who have experienced cholestatic jaundice of pregnancy. Patients with a history of cholestatic jaundice of pregnancy should be carefully observed during ORTHO-NOVUM 1/80□21 or ORTHO-NOVUM 1/80□28 therapy.

Adverse reactions observed in patients receiving oral contraceptives: A statistically significant association has been demonstrated between use of oral contraceptives and the following serious adverse reactions: thrombophlebitis, pulmonary embolism, cerebral thrombosis and hemorrhage, myocardial infarction, gallbladder disease.

Although available evidence is suggestive of an association, such a relationship has been neither confirmed nor refuted for the following serious adverse reactions: neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis, hepatic lesions with or without intra-abdominal bleeding.

The following adverse reactions are known to occur in patients receiving oral contraceptives: nausea, vomiting, gastrointestinal symptoms (such as abdominal cramps and bloating), breakthrough bleeding, spotting, change in menstrual flow, amenorrhea during and after treatment, edema, chloasma or melasma, which may persist, cholestatic jaundice, migraine, rash (allergic), mental depression, change in weight (increase or decrease), breast changes (tenderness, enlargement and secretion), changes in cervical erosion and cervical secretions, suppression of lactation when given immediately post-partum, rise in blood pressure in susceptible individuals.

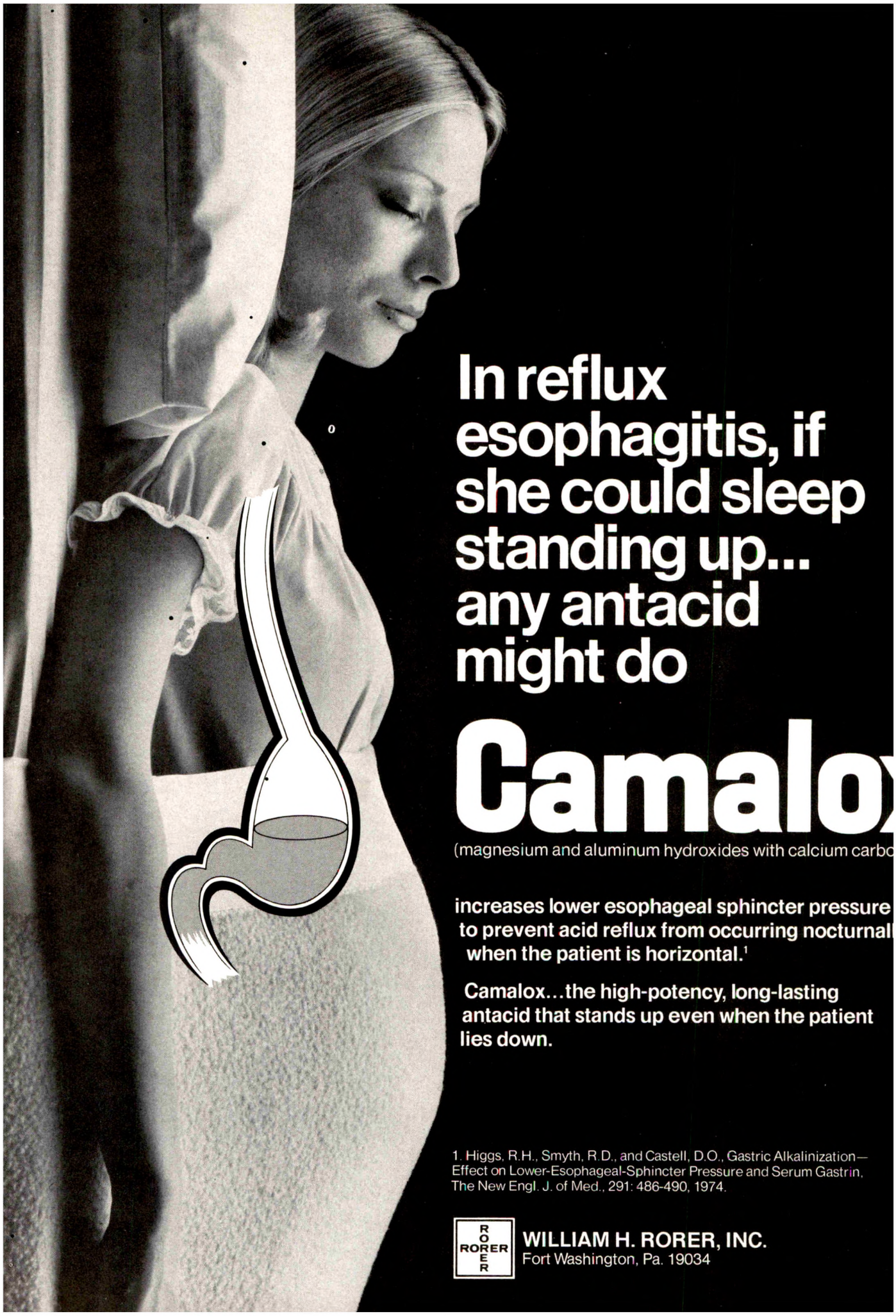
Although the following adverse reactions have been reported in users of oral contraceptives, an association has been neither confirmed nor refuted: anovulation post-treatment, which tends to occur more frequently in women with a history of menstrual disorders, premenstrual-like syndrome, changes in libido, changes in appetite, cystitis-like syndrome, headache, intolerance to contact lenses, nervousness, dizziness, fatigue, backache, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruptions, itching, vaginitis.

The following laboratory results may be altered by the use of oral contraceptives: hepatic function—increased sulfobromophthalein retention and other tests; coagulation tests—increase in prothrombin, Factors VII, VIII, IX and X; decrease in anti-thrombin III; increase in platelet aggregability; thyroid function—increase in PBI, and butanol extractable protein-bound iodine and decrease in T^3 uptake values; metyrapone test; pregnanediol determination.

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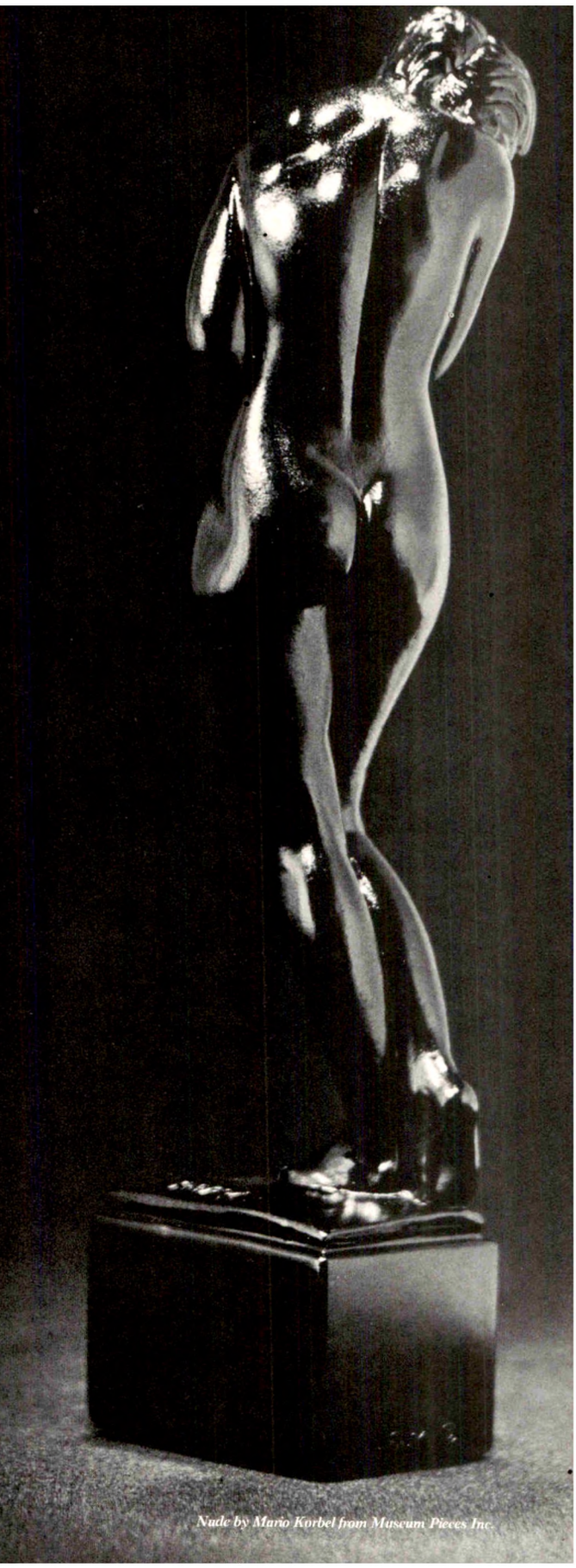
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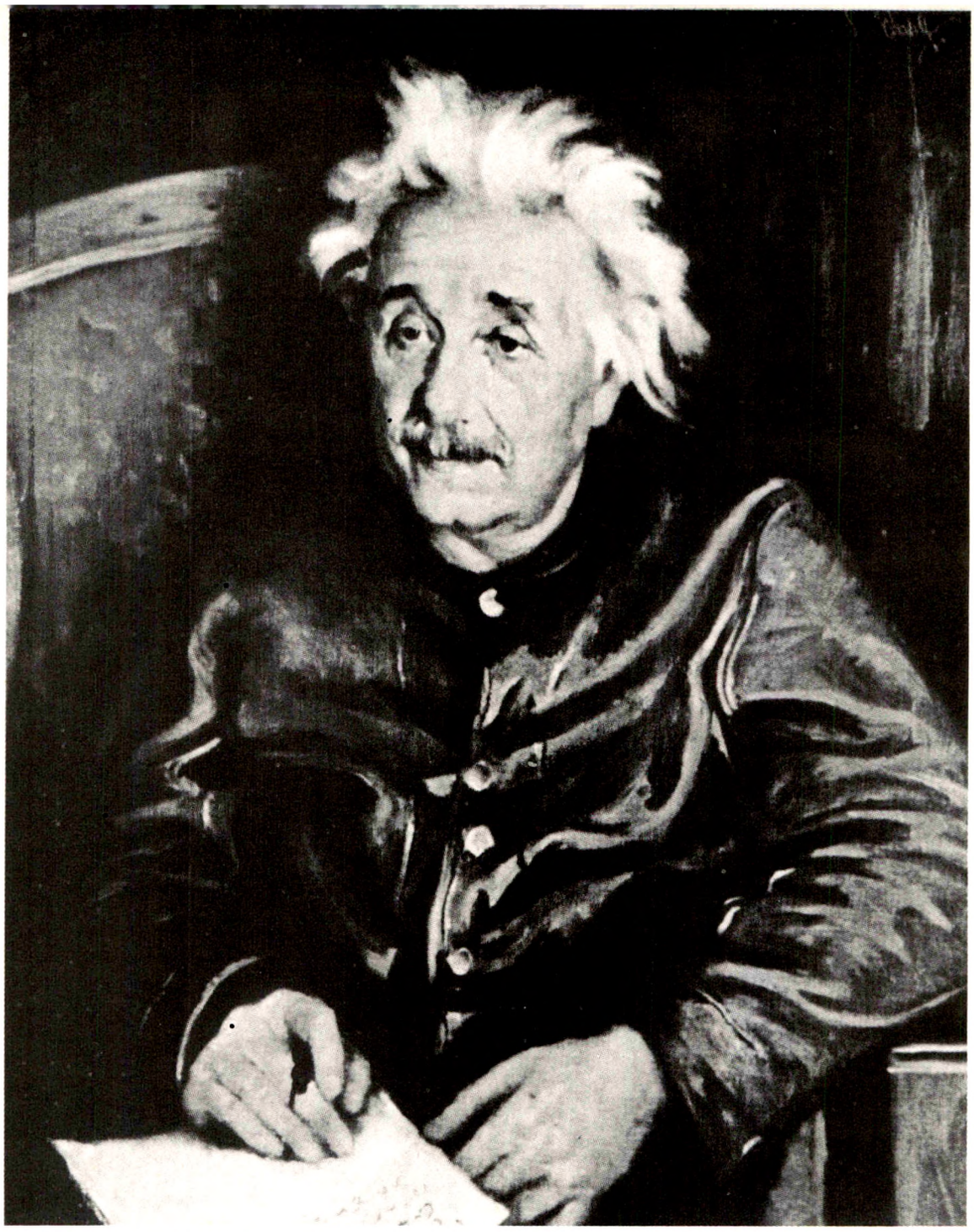
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OBSTETRICS

The influence of aspirin on the course of induced midtrimester abortion

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Prostaglandins appear to mediate the uterine contractions of abortion and labor, and aspirin has been shown to be an inhibitor of prostaglandin synthesis. In this double-blind, placebo-controlled, prospective, and randomized study, aspirin was administered orally in doses of 600 mg. every 6 hours to patients undergoing induced midtrimester abortions with hyperosmolar urea and oxytocin augmentation. The mean injection-abortion interval was significantly prolonged by aspirin in nulliparous patients (aspirin 32.3 ± 3.3 hr. vs. placebo 21.5 ± 3.5 hr.) and no aspirin-treated nullipara aborted in less than 18 hours. There was no significant difference between treatment groups in the mean injection-abortion interval in the multiparous patients at the dose of aspirin used. The effectiveness of aspirin in the prolongation of the injection-abortion interval has potential therapeutic implications for the treatment of premature labor.

SEVERAL AUTHORS[1–3] have speculated that prostaglandins may mediate the uterine contractions of abortion and labor. Prostaglandins have been found to be low or absent from amniotic fluid and blood of patients at all stages of pregnancy who are not in labor.[2, 4, 5] Prostaglandins are present in high concentrations, however, in amniotic fluid and blood of patients during labor and spontaneous abortion,[2, 4, 5] and are released into the amniotic fluid after extra-amniotic injection of hypertonic saline for induction of abortion.[1] In addition, inductions of abortion and of labor at term have been successfully accomplished by infusions of prostaglandins.[6, 7] These observations suggest that prostaglandins may have an important role in abortion and labor.

Aspirin has been shown to block the production and release of prostaglandins in vitro in guinea pig lung,[8] in human platelets,[9] and in dog spleen.[10] In one retrospective study,[3] patients taking high-dose salicylate during pregnancy have been found to have a significant increase in the average length of gestation, in the frequency of postmaturity, and in the mean duration of spontaneous labor, although some of these patients had underlying diseases and were not screened for other drugs which might have influenced

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Table I. Effect of aspirin on the course of induced midtrimester abortion in multiparous patients*

	Placebo	*Aspirin*
Number	12	12
Maternal age (yr.)	22.8 ± 0.9	24.4 ± 1.3
Gestational age (wk.)	18.6 ± 0.5	19.3 ± 0.4
Est. blood loss (ml)	354 ± 66	246 ± 65
Inject.-ab. interval (hr.)	20.0 ± 3.9	17.1 ± 2.5
Inject.-ab. interval, range (hr.)	6.5 – 43.2	6.8 – 30.8

*Values are means ± S.E.M.

Table II. Effect of aspirin on the course of induced midtrimester abortion in nulliparous patients*

	Placebo	*Aspirin*
Number	12	12
Maternal age (yr.)	18.8 ± 0.8	18.7 ± 0.7
Gestational age (wk.)	18.5 ± 0.7	19.3 ± 0.4
Est. blood loss (ml.)	274 ± 61	410 ± 82
Inject.-ab. interval (hr.)	21.5 ± 3.5	32.3 ± 3.3†
Inject.-ab. interval range (hr.)	7.7 – 38.5	18.0 – 51.3

*Values are means ± S.E.M.
†Significantly different from placebo at $p < 0.05$ (t test).

the above-mentioned parameters. It has been previously shown that in patients undergoing hypertonic saline–induced midtrimester abortion, oral administration of indomethacin or acetylsalicylic acid prolongs the injection-abortion interval.[11]

The present study was undertaken to determine the effect of oral administration of acetylsalicylic acid in low doses on the injection-abortion interval in patients undergoing hyperosmolar-urea–induced midtrimester abortion. These patients also received continuous oxytocin infusion during the injection-abortion interval.

Patient population

The study group was selected from those patients who had already been approved for midtrimester abortions and who gave informed written consent to participate. All patients had hematocrit and stool guaiac determinations. A screening urinalysis for salicylates, alcohol, and the common drugs of abuse was performed for each patient. Disqualifying characteristics included: positive urinalysis for salicylates, alcohol, or the common drugs of abuse; admitted use of any drug within the 7 days before amniocentesis; history of sensitivity to salicylates; hematocrit less than 30 per cent; positive stool guaiac; history of peptic ulcer disease.

Study design

All subjects received an intra-amniotic injection of 80 Gm. of urea as a 58 per cent solution in 5 per cent dextrose in water and all received a continuous intravenous infusion of oxytocin at 333 mμ per minute until the abortion was complete. The patients were studied in two groups, nulliparous and multiparous. A double-blind, randomized, prospective study was done with two treatment groups—aspirin and placebo. The selection of the treatment group was according to a table of random numbers in the order in which the patients presented. Patients were given hard gelatin capsules containing either 300 mg. of aspirin or lactose as a placebo. The dosage regimen of two capsules orally every 6 hours was started approximately 1 hour before the urea injection and continued until the time of abortion or until 30 hours after the first dose, whichever occurred first. Immediately before the amniocentesis, maternal blood and urine samples were obtained for determination of salicylate levels,[12] and the urine specimen was analyzed qualitatively for the presence of other drugs.[13] Amniotic fluid aspirated just before the urea was injected was also analyzed for salicylate. A second blood salicylate level was obtained at 12 hours, just before the third dose of aspirin. No patient had vaginal bleeding or nausea sufficient to necessitate discontinuation of the study drug.

The aspirin-treated group was compared with the placebo group with respect to the following parameters: maternal age, gestational age, injection-to-abortion interval, total estimated blood loss, incidence of complications, and analgesic requirements.

Gestational age was determined from a chart relating fetal foot length to gestational age.[14] Blood loss was estimated by adding the weights (over tare) of all perineal pads used during the abortion procedure and up to 2 hours after completion of the abortion.

Results

Results for multiparous patients are shown in Table I and for nulliparous patients in Table II. There were no statistically significant differences in the means of maternal age or gestational age between treatment groups. Although there was a tendency for increased blood loss in the aspirin-treated nulliparas, the difference in mean blood loss between the two groups was not statistically significant by t test. There was no significant difference in the mean injection-abortion interval between treatment groups in the multiparous patients, but in the aspirin-treated nulliparous group there was a significant prolongation of the mean injection-abortion interval ($p < 0.05$, t test). The

shortest interval among the aspirin-treated nulliparas was 18 hours, whereas seven out of 12 of the placebo-treated patients had intervals of less than 18 hours ($p < 0.01$, chi square). Fig. 1 illustrates the mean injection-abortion intervals in all four groups.

Amniotic fluid salicylate levels at the time of amniocentesis ranged from nondetectable to 2.0 mg. per 100 ml., with a mean of 0.4 mg. per 100 ml. for all aspirin-treated patients. The mean blood salicylate level at the time of urea injection was 4.9 ± 0.5 mg. per 100 ml. for the nulliparous patients, and 4.3 ± 0.7 mg. per 100 ml. for the multiparous patients. At 12 hours after injection the levels were 5.6 ± 0.8 mg. per 100 ml. for the nulliparous group and 4.7 ± 0.5 mg. per 100 ml. for the multiparous group. The tendency for blood salicylate levels to correlate positively with the injection-abortion intervals was not statistically significant by t test.

Complications were few in each group. There was one case of endometritis and one transverse cervical laceration in the placebo group. One patient in the aspirin group presented later with retained tissue which required curettage. There was no difference in analgesic requirements between treatment groups.

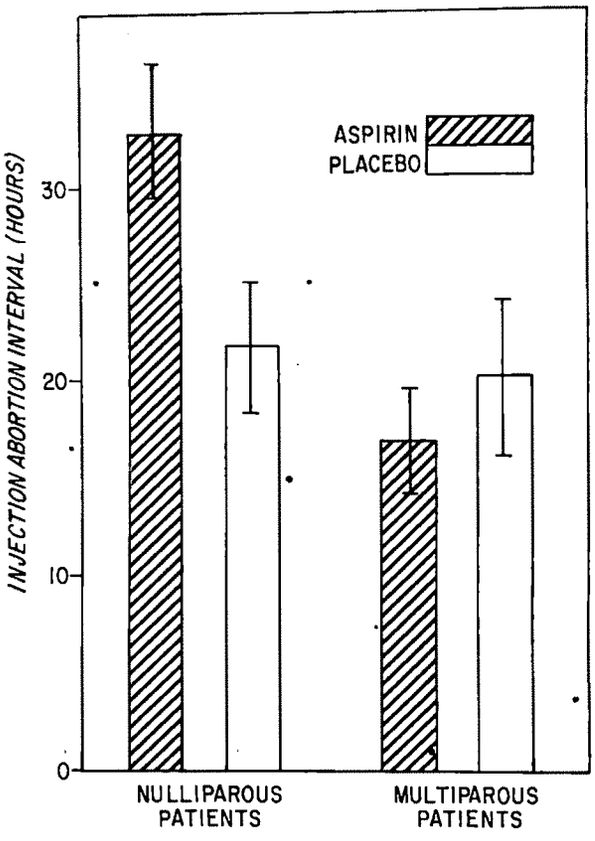


Fig. 1. Effect of aspirin (600 mg. every 6 hours postoperatively) on the mean injection-abortion interval after intra-amniotic instillation of hyperosmolar urea. Intravenous oxytocin infusion was administered throughout the injection-abortion interval. Aspirin-treated nulliparas but not multiparas had a significantly longer interval than placebo-treated controls.

Comment

The results of this study demonstrate that in nulliparous patients, relatively low doses of aspirin can delay the injection-abortion interval in patients undergoing induced midtrimester abortion with intra-amniotic hyperosmolar urea and continuous intravenous oxytocin. The mean injection-abortion interval was prolonged, and no aspirin-treated patient aborted in less than 18 hours. It may be that higher doses of aspirin would be effective in multiparous patients. It is possible that the higher cervical resistance in nulliparous patients makes them more susceptible to prolongation of the injection-abortion interval by inhibition of uterine activity. Perhaps at these dosage levels the aspirin effect was not sufficient to overcome that of oxytocin in the multiparous patient. In the study of Waltman, Tricomi, and Palav,[11] the effect of parity was not evaluated, yet aspirin at the same dosage used in the present study significantly prolonged the saline-induced abortion in women of all parities combined. It should be noted, however, that these investigators did not administer oxytocin to all patients, reserving it for certain indications.

Experimental evidence suggests that aspirin and other nonsteroidal acidic anti-inflammatory drugs exert their pharmacologic effects through inhibition of prostaglandin formation.[8–10] The results of the present study contribute to the growing body of evidence which suggests that prostaglandin formation is an important determinant in the development of strong and coordinated uterine contractions in a variety of obstetric situations. Furthermore, these results support the hypothesis that hyperosmolar agents such as urea exert their abortifacient effect through a prostaglandin-dependent mechanism. Thus, patients included in studies of abortifacients should be screened for usage of drugs which may inhibit prostaglandin formation. In this regard the recent report of MacDonald and associates[15] is of interest. They induced midtrimester abortions in 10 of 12 patients with intra-amniotic injections of arachadonic acid, a prostaglandin precursor, but it is of note that both nonresponders had recently ingested aspirin.

The effectiveness of aspirin in the inhibition of uterine contractions has potentially important therapeutic implications for the treatment of premature

labor. Recommendations of aspirin for this purpose must await clinical trials of its efficacy and safety.

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The obstetrician's role in reducing the risk of aspiration pneumonitis

With particular reference to the use of oral antacids

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Aspiration of gastric contents is the commonest cause of maternal anesthetic death. If the pH of the contents is below 2.5, morbidity and mortality rates are likely to be higher than if the pH is above 2.5. A study of 146 patients in labor shows that one in four patients is "at risk" to acid aspiration. No time interval between last meal and either onset of labor or delivery can guarantee an empty stomach. The use of oral antacids during labor reduces the number at risk to 1 in 35. Other methods by which the obstetrician may contribute to reducing the risk are discussed.

ACID ASPIRATION pneumonitis is usually considered an anesthetic complication. This apportionment of blame, however, has detracted from a full appreciation of the role that can be played by the obstetrician in its prevention. The role of gastric acidity in the etiology of aspiration pneumonitis has been clearly established.[1–3] Recently, the value of oral antacid administration in significantly neutralizing gastric acidity has been conclusively demonstrated.[4] As the logistics of obstetric practice in the United States often excludes the anesthesiologist, at least until delivery is imminent, the required prophylactic measures demand the full involvement of the obstetrician.

The etiology of aspiration pneumonitis

Hall[5] in 1940 drew attention to the frequency of aspiration pneumonitis in obstetrics. It is interesting to note in passing that he entitled his paper "Aspiration pneumonitis: an *obstetric* hazard." Of the 14 cases he reported, three were during cesarean section. The syndrome of acid aspiration is now associated with the name of Curtis Mendelson,[1] who, in a classic paper in 1946, showed that 96 per cent of 66 cases of aspiration involved acid gastric contents. In animal experiments reported in the same paper, he clearly demonstrated that the primary etiologic factor in aspiration pneumonitis was the acidity of the aspirate and that a pH of below 2.5 was required to produce the pathognomonic picture of cyanosis, tachycardia, dyspnea, and expiratory wheezing which could often lead to death. Other workers[2, 3] have confirmed the importance of this low pH and have shown that pepsin and trypsin alone have minimal effects on this tracheobronchial tree. Gastric juice neutralized to a pH of above 2.5, if aspirated, does not produce the syndrome.

The exact volume of acid gastric content required to produce the acid aspiration syndrome in human subjects is unknown. However, 1 ml. per kilogram of acid gastric juice injected into the trachea in dogs will produce the syndrome; increasing this dose does not significantly add to the severity of the pneumonitis.[6] More recent, as yet unpublished, work in the rhesus monkey by Roberts and Adamsons shows that doses over 0.4 ml. per kilogram can produce significant changes in the lung, though not necessarily fatal to the animal. This translates approximately to 25 ml. in the adult human female and based on this we have suggested that a patient "at risk" be defined as that patient with at least 25 ml. of gastric juice of pH below 2.5 in the stomach at delivery.[4]

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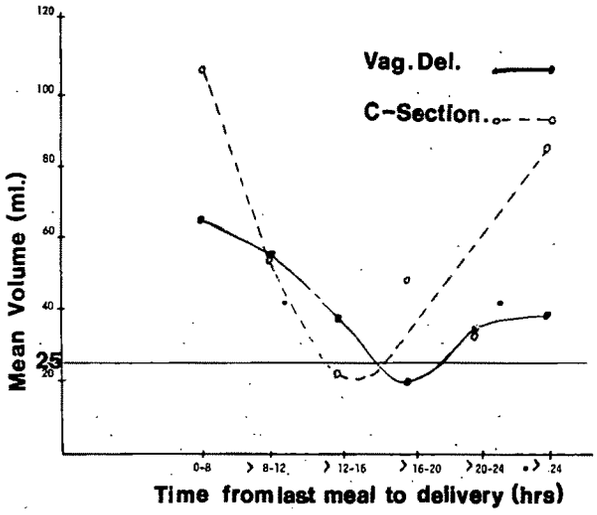


Fig. 1. Mean gastric content volume aspirated at delivery in 146 patients in labor related to the time between last meal and delivery.

Prophylaxis by oral antacids

Any method, therefore, which either reduces gastric content volume to below 25 ml. or raises gastric pH to above 2.5 can reduce the number of patients at risk. In his original paper Mendelson suggested the use of warm antacids both as an emetic and a buffer for residual acid. In 1966 Taylor and Pryse-Davies[7] recommended administration of oral antacids during labor and the practice gained some acceptance in the United Kingdom and a few other countries. Unfortunately, the United States was not among these countries until 1974, when Roberts and Shirley[4] showed conclusively that the use of antacids prior to cesarean section reduced the number of patients at risk from one in four to one in 48. A highly significant feature of this work was the recognition that patients for elective cesarean section were equally, if not more, at risk than patients for emergency cesarean section. As the possibility exists that patients who deliver vaginally may somehow demonstrate different gastric physiology from those whose labor ends in cesarean section, a further study has been undertaken. The purpose of the study was to try to identify those patients in labor at risk to aspiration pneumonitis and to assess the value of oral antacids in maintaining the gastric pH level above 2.5.

Method

A total of 146 consecutive patients in labor receiving general anesthesia when one or other of the authors was physically present were studied. Of these, 70 were given 15 ml. of an oral mixture of hexitol-stabilized magnesium and aluminum hydroxide* within 4 hours of delivery, 55 received no oral antacid, and 21 were given the antacid more than 4 hours prior to delivery. There were 82 patients who delivered vaginally and 64 who underwent cesarean section. (Oral antacids are now given routinely every 3 hours at our institution. At the start of our study, however, their administration was ordered only by one of the authors; patients who received no antacid in this study were those for whom the authors were not present in time to order it.) Each patient was preoxygenated before induction with sodium thiopental and endotracheal intubation was performed after relaxation with succinylcholine. Head-up position and cricoid pressure were utilized in all cases. Anesthesia was maintained with nitrous oxide and oxygen, occasionally 0.5 per cent methoxyflurane being added. For cesarean section further doses of succinylcholine or d-tubocurarine were used as required. Gastric aspiration was always performed prior to the administration of d-tubocurarine due to its known ability to release histamine. After intubation a No. 18 Levin tube was passed into the stomach, which was emptied as far as possible. The volume of gastric contents aspirated was measured and the pH estimated.† The color of gastric contents ranged from clear to deep green. The latter, suggesting the presence of bile, was not always associated with a high pH. Small portions of undigested food material were often present (large particles obviously could not be aspirated through a No. 18 Levin tube). On occasion, a watered-down suspension of the antacid itself was retrieved. It is impossible to tell whether the stomach had been completely emptied by this aspiration; all volumes expressed in the results are thus minimum volumes present, though they are expressed as the maximum volumes obtained. Time of last meal (ignoring sips of water taken later), time of onset of labor contractions, and time of antacid administration were recorded. Also noted were parity, maternal weight, infant's condition, and other drugs used during labor.

Results

Fig. 1 to 4, showing the mean and maximum volumes of gastric contents aspirated related to both the time from the last meal to delivery and to the onset of labor, demonstrate that no time interval since the last meal guarantees a low volume of juice in the stomach. Patients who ate *after* labor started, irrespec-

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†I.L. Labomatic, Instrumentation Laboratories, Inc., Lexington, Massachusetts.

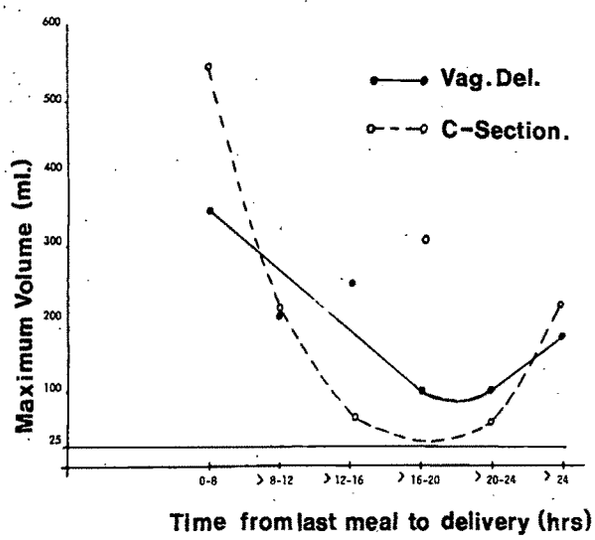


Fig. 2. Maximum gastric content volume aspirated at delivery in 146 patients in labor related to the time between last meal and delivery.

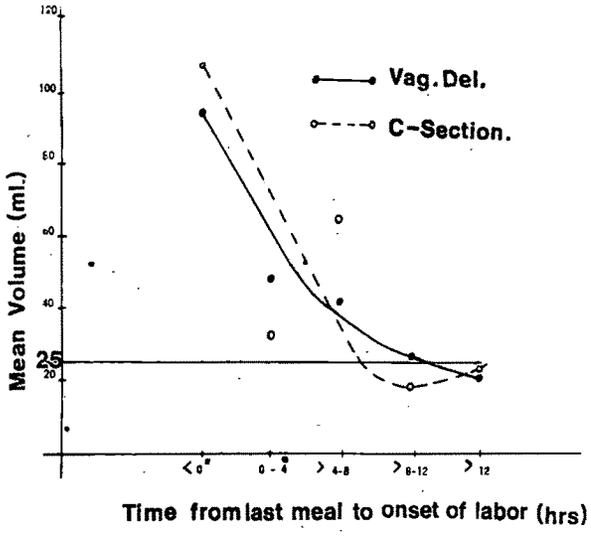


Fig. 3. Mean gastric content volume aspirated at delivery in 146 patients in labor related to the time between last meal and onset of labor. Asterisk indicates patients who ate after labor started.

tive of the time in labor, showed exceptionally high volumes. Though mean gastric volume content decreases with the time since the last meal (at least up to 16 to 20 hours) no time interval guarantees a gastric volume of below 25 ml. The volume curves also demonstrate that there is no significant difference in the volume of gastric juice likely to be in the stomach at delivery whether labor progresses to vaginal delivery or terminates in cesarean section.

Table I shows that, while 13 out of 55 patients who received no antacid were, by our definition, "at risk," only two patients out of 70 who had received antacid within 4 hours of delivery could be so classified. When the antacid had been given more than 4 hours before delivery, five out of 21 patients were still "at risk." The difference in the number at risk between those patients receiving their antacid within 4 hours of delivery and the others is highly significant.

A previous series[8] of elective cesarean sections has shown similar results: seven out of 24 patients receiving no antacid were "at risk" and of 31 who did receive antacid none was "at risk."

If pH alone is considered (without reference to volume) 40 per cent of patients who received no antacid, 48 per cent of patients who received antacid more than 4 hours before delivery, and 10 per cent of patients who did receive antacid within 4 hours of delivery had a pH of below 2.5. No significant difference could be detected between those who delivered vaginally and those by cesarean-section.

As acid aspiration pneumonitis is due to a combination of pH and volume, we believe that the results shown in Table I, considering both factors, give a more realistic appraisal of the value of oral antacids.

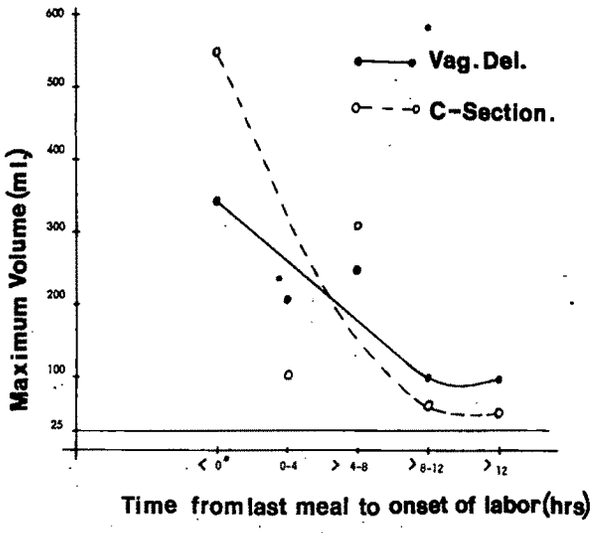


Fig. 4. Maximum gastric content volume aspirated at delivery in 146 patients in labor related to the time between last meal and onset of labor. Asterisk indicates patients who ate after labor started.

Fifty per cent of patients whose last meal was within 12 hours of delivery and 33 per cent of patients who had eaten more than 12 hours before delivery had a pH of below 2.5 if no antacid had been given. Four out of six patients whose last meal was more than 20 hours before delivery and who had received no antacid had a pH below 1.8. As mean volume increases also in

Table I. Number of patients "at risk" (volume of gastric aspirate > 25 ml., pH of gastric aspirate < 2.5)

*Antacid**	*In labor*				*Elective cesarean section†*
	Cesarean section†	*Vag. del.*	*Total*		
Within 4 hr. of delivery	1 (3%) n = 31	1 (2.5%) n = 39	2 (3%) n = 70		0 (0%) n = 31
More than 4 hr. before delivery	4 (36%) n = 11	1 (10%) n = 10	5 (24%) n = 21	18 (24%) n = 76	—
No antacid	4 (18%) n = 22	9 (28%) n = 33	13 (24%) n = 55		7 (29%) n = 24

*WinGel 15 ml.
†For comparison (from Roberts and Shirley.[8])

Table II. Influence of weight on mean gastric content volume at delivery

	>160 lb.	*<160 lb.*
In labor	82 ml (n = 45)	26 ml (n = 79)
Not in labor	17 ml (n = 20)	15 ml (n = 29)

Table III. Influence of scopolamine during labor on mean gastric content volume and pH

	With scopolamine	*No scopolamine*
Mean volume (ml.) (all patients in labor)	38 (n = 47)	51 (n = 99)
pH (mean and range) (only patients receiving no antacid)	3.51(1.3-7.88) (n = 27)	2.98(1.35-6.6) (m = 28)

patients fasting for over 20 hours, it would appear that such patients are particularly at risk and that the comment "she has not eaten since yesterday" should be taken as a warning rather than as reassurance.

Patients in labor who weighed over 160 pounds had significantly higher volumes of gastric juice at delivery than those who weighed less (Table II). As this did not hold true for the patients who underwent elective cesarean section, weight does not seem to be an intrinsic cause but rather a reflection on the eating habits of such patients, many of whom had eaten after the onset of labor. The use of scopolamine during labor had no significant effect on either volume or pH (Table III).

While it would seem reasonable to believe that the administration of antacids during labor would in itself increase gastric content volume, the mean volume of

Table IV. Mean gastric content volume at delivery in 146 patients in labor

Antacid	*Volume*	
Within 4 hr.	44 ml. (n = 70)	45 ml. (n = 91)
More than 4 hr.	49 ml. (n = 21)	
None given		48 ml. (n = 55)

those patients who received antacid was, in fact, no different from that of those who did not (Table IV). Raising the pH of intragastric contents is known to have other effects, such as an increase in tone at the cardioesophageal junction;[9] this in itself may decrease the likelihood of regurgitation. Fig. 5 shows that provided the antacid is given within 4 hours of delivery few patients exhibit a pH of below 2.5. In those patients receiving oral antacid more than 4 hours before delivery 48 per cent had a pH of below 2.5 and five out of 21 could be defined as "at risk." Though these figures are too small for definite conclusions, they suggest that not only was the antacid ineffective but that acid rebound, which might actually increase the risk, occurred.

Comment

In 1968, of 950 maternal deaths in the United States, 8 per cent were estimated to be due to anesthesia,[10] establishing it as the fourth largest cause of maternal death after hemorrhage, toxemia, and infection. In 1951 Merrill and Hingson[11] estimated that 100 maternal deaths a year were due to aspiration; in 1961 Phillips and his co-workers[12] estimated an average of one maternal aspiration death a year for each community of one million people.

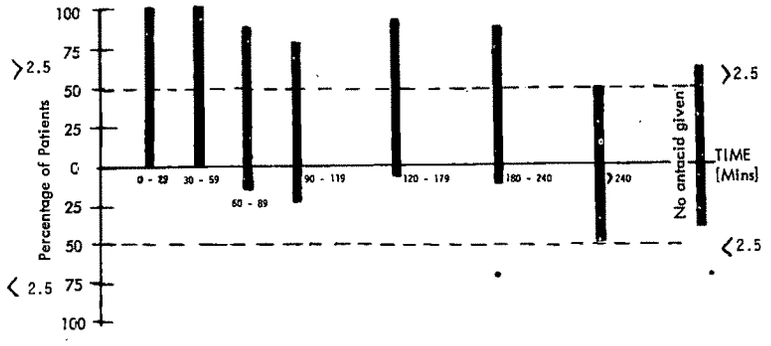


Fig. 5. Percentage of patients with gastric pH above and below 2.5 related to the time since administering antacid orally (n with antacid = 91, n without antacid = 55).

Table V.* Steps that have been recommended to minimize risks of aspiration in parturients, and disadvantages thereof (from various sources)

Recommended step	*Disadvantage*
1. NPO during labor	Patient may eat before admission. Gastric contents increased during long labor
2. Preanesthetic emptying of stomach by	
a. N-G tube	Theoretically ideal; unpleasant; effectiveness doubtful; time-consuming. False sense of security
b. Apomorphine	Circulatory collapse reported. Undiagnosed esophageal pouch or hiatus hernia possible
3. Awake intubation	Barbaric without local anesthesia. Vomiting and aspiration possible if laryngeal reflexes obtunded by local anesthetic
4. Head-up or anti-Trendelenburg	Intubation difficult. If regurgitation or vomiting occurs, aspiration inevitable. Intragastric pressure high after succinylcholine
5. Cricoid pressure	Skilled assistant needed. Rupture of esophagus with vomiting
6. Head-down and/or inhalational induction	Flammable agents often used. Fetal depression. Unprotected trachea
7. Lateral position	Obstructed airway enhances vomiting
8. Mandatory endotracheal intubation	Inexperienced intubators not always successful. Aspiration still possible before, during, and after intubation
9. Oral antacids	May not be effective with large gastric volumes
10. Regional anesthesia	Aspiration still possible, especially if patient is also sedated. Other complications may be higher than with general anesthesia

*Reprinted with permission from Roberts, R. B., and Shirley, M. A.: Anesthesia and Analgesia **53**:859, 1974.

The system of classification of deaths in the United States does not show the precipitating factor which led to the terminal illness. Therefore, it is difficult to assess accurately the percentage of maternal anesthetic deaths due to aspiration of gastric contents. Studies from the United Kingdom, however, show that over half of all such deaths are due to this cause. The report on confidential inquiries into maternal deaths in England and Wales[13] from 1967 through 1969 incriminated anesthesia in 7.2 per cent of maternal deaths. Of the 50 anesthetic deaths reported during this period, 26 were due to inhalation of gastric juice. Thirty-six anesthetic deaths were associated with cesarean section, of which 24 had an "avoidable factor."

The incidence of cesarean section is increasing; in the United States it has now reached over 7 per cent and in teaching institutions is often double this figure. The safety of modern anesthesia is sometimes quoted as a factor influencing the choice of cesarean section in some patients and in view of this fact the increase in anesthetic deaths is particularly ominous.

The obstetrician's role. Many of the methods recommended for decreasing the incidence of aspiration during delivery together with some of their disadvantages are listed in Table V. Many of these relate to anesthetic techniques but many refer to items directly under the obstetrician's control. The primary blame for any inadequacies in obstetric anesthesia lies with the difficulty in obtaining anesthetic staff especially for obstetric coverage. In the United States a report of The Commission on Maternal Health[14] in 1970 found that 30 per cent of hospitals had no M.D. coverage at all and 22 per cent no nurse anesthetist coverage. Only 6 per cent of hospitals had M.D. coverage for the full 24 hours and only a further 14

Table VI. Summary of recommendations by which the obstetrician can reduce acid aspiration pneumonitis

1. Emphasize to *every* patient the importance of not eating or drinking once labor has started
2. Administer to *every* patient 15 ml. of an oral antacid every 3 hr. during labor or 1 hour prior to elective cesarean section
3. Encourage the use of local and regional anesthetic techniques
4. Do not discourage the anesthesiologist from endotracheal intubation during general anesthesia
5. Remember that *any* obstetric patient may have a full stomach irrespective of the time since the last meal
6. Acquire expertise in intubation and ventilation if you administer your own regional blocks. Ensure the presence of a second physician to monitor such blocks during the delivery
7. Insist on the same standard of anesthetic care and coverage for obstetric patients as you do for gynecologic patients

per cent had CRNA coverage. Obstetricians can and should exert more pressure both on their department of anesthesiology and on the administrators of their hospital to provide personnel and funds for the proper care of maternity patients requiring anesthesia. "Since the obstetrician is the primary physician for the parturient, he has a major responsibility in making adequate anesthesia coverage available."[15]

Even if a patient is scheduled for prepared childbirth or for regional anesthesia, the emergency situation or the failed regional may call for a general anesthetic in any parturient. The significantly higher volume of gastric juice present in those patients who ate after labor started (Figs. 3 and 4) is an overwhelming argument for the obstetrician emphasizing the extreme importance of the patient not eating if she even suspects that she is in labor. Particular attention should be paid to this point during prenatal visits rather than a mere passing mention.

The increased popularity of prepared childbirth methods and the use of pudendal block and local infiltration contribute significantly to a reduction in anesthetic morbidity and deaths. Obstetricians can encourage this trend. Bowing to the wishes of a patient to be "completely out" during labor and delivery carries a serious risk which few patients fully appreciate. It is incumbent upon the obstetrician to appraise the patient of this risk. See the recommendations in Table VI.

Regional anesthesia dramatically reduces the risk of aspiration pneumonitis; this risk, however, is not totally eliminated.[16] Contrary to popular belief the presence of active laryngeal reflexes is not a 100 per cent guarantee against aspiration. If the reflexes are partially obtunded by sedation or if the regurgitation is massive the risk is increased. Regional anesthesia also has its own complications, mainly hypotension and total spinal anesthesia. It is impossible for an obstetrician to monitor adequately regional anesthesia instituted by himself and devote his full attention to the delivery of the infant. The presence of a second physician, or, at least, a fully trained nurse with no other duty in the delivery room, should be mandatory. While it is appreciated that many obstetricians are highly skilled in the use of spinal, caudal, or epidural techniques they should be equally trained and prepared for emergency procedures such as endotracheal intubation, ventilation, and external cardiac assistance.

Though endotracheal intubation does not guarantee that aspiration cannot occur either immediately prior to intubation or following extubation, it nevertheless adds considerably to the safety of a general anesthetic. The competent anesthesiologist, who feels that intubation is indicated in anesthetizing a patient who may have a significant volume of highly acid gastric juice, should not be discouraged by the obstetrician, whose concern about postintubation sore throat is usually exaggerated. Even the 5 to 8 per cent of patients who may be expected to have a sore throat for the first 24 hours are more likely to complain about their perineal sutures. Induction and intubation should be performed before the patient is placed in the lithotomy position, as this position increases intragastric pressure significantly.

Analgesic concentrations of nitrous exide, trichlorethylene, or methoxyflurane, combined with local infiltration or pudendal block may be administered to patients without their losing either consciousness or protective reflexes. These patients rarely, if ever, require intubation though, naturally, facilities for suction and intubation must be at hand. If general surgical anesthesia is induced, however, by either intravenous or inhalation agents, we feel that these patients should be intubated. In the ACOG Technical Bulletin No. 11, February 1969 (revised October 1973), on p. 2 appears the following statement: "2. If, however, general anesthesia is medically indicated for delivery, a cuffed endotracheal tube should be inserted."

Finally, the benefits of oral antacid therapy are now beyond doubt. Unpublished animal work by Roberts and K. Adamsons, Jr., shows that aspiration of the antacid itself or of gastric juice neutralized by the antacid causes no more than temporary hypoxia from obstruction and has no irritant effect on the tracheobronchial tree. We suggest a 3 hour regimen of oral antacid administration during every labor; this appears

logistically feasible and yet is well within the 4 hour deadline. Most labors are managed by obstetricians; the anesthesiologist, if he participates at all, is often called immediately prior to delivery. The institution of routine oral antacid prophylaxis will rest in most cases with the obstetrician.

As this paper is concerned with prevention of aspiration, a detailed description of the treatment is not relevant. Based on this work, however, we do suggest one refinement to the usual methods of treatment when aspiration is known or suspected to have occurred. A sample of any residual gastric contents should be obtained and the pH estimated. If the pH is above 2.5, steroids are unlikely to be of any benefit to the patient. If the pH is below 2.5, serious consideration should be given to maintaining controlled ventilation for a number of hours postoperatively until the clinical picture is clarified.

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Effect of estradiol-17β on blood flow to reproductive and nonreproductive tissues in pregnant ewes

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The effect of estradiol-17β (1 μg per kilogram) on regional blood flow and cardiac output was studied by means of radionuclide-labeled microspheres in 6 nonpregnant and 13 pregnant ewes five to seven days after operation. Estradiol caused vasodilation in myometrium, endometrium, and placental cotyledons throughout pregnancy, but these responses were significantly less than the fifteenfold increase seen in the nonpregnant uterine tissues. Significant vasodilation also occurred in the ovaries, cervix, vagina, uterine tubes, mammary gland, skin, and adrenal glands of pregnant ewes. Cardiac output increased by 14 per cent. No significant change in uterine oxygen consumption was associated with the increase in blood flow to the pregnant uterus.

DURING THE COURSE of pregnancy there are large increases in placental, myometrial, and endometrial flows.[14] The factors responsible for these changes are unknown.[12] Indirect evidence supporting the hypothesis that estrogens play an important role is derived from the considerable increase of uterine blood flow following the intravenous injection of 1 μg per kilogram of estradiol-17β (E_2) in nonpregnant, oophorectomized ewes[5] and marked dilation by E_2 of the arterioles of the uterine caruncles, later to be the arterioles of the placental cotyledons in the sheep.[13] Furthermore, Greiss and Marston[3] have reported a 43 per cent increase in the uterine blood flow of ewes in the last third of pregnancy following the systemic infusion of exogenous estrogens. These observations raise the question whether placental blood flow can be increased by the administration of estrogens.

It is the purpose of this report to describe the circulatory changes caused by the intravenous injection of E_2 in sheep in various stages of gestation. The microsphere method was used in order to determine the blood flow to different organs and tissues before and after estrogen stimulation.

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Material and methods

Six nonpregnant and 15 pregnant Western ewes with gestations from 38 to 139 days were studied. Two of the pregnant ewes, at 90 and 140 days of gestation, respectively, were not able to stand up after operation, appeared sick, and showed a progressive decline of uterine blood flow over a five-day period. These two ewes have been excluded from the Results section.

Experimental design. The biological preparation consisted of ewes with electromagnetic flow probes implanted around both uterine arteries and indwelling catheters in the following locations: (1) both femoral arteries, (2) the high inferior vena cava via a femoral vein, (3) the left ventricle via the left common carotid artery, and (4) the major vein draining each side of the uterus in the pregnant animals. All catheters and flow probes were placed as one surgical procedure. Post-

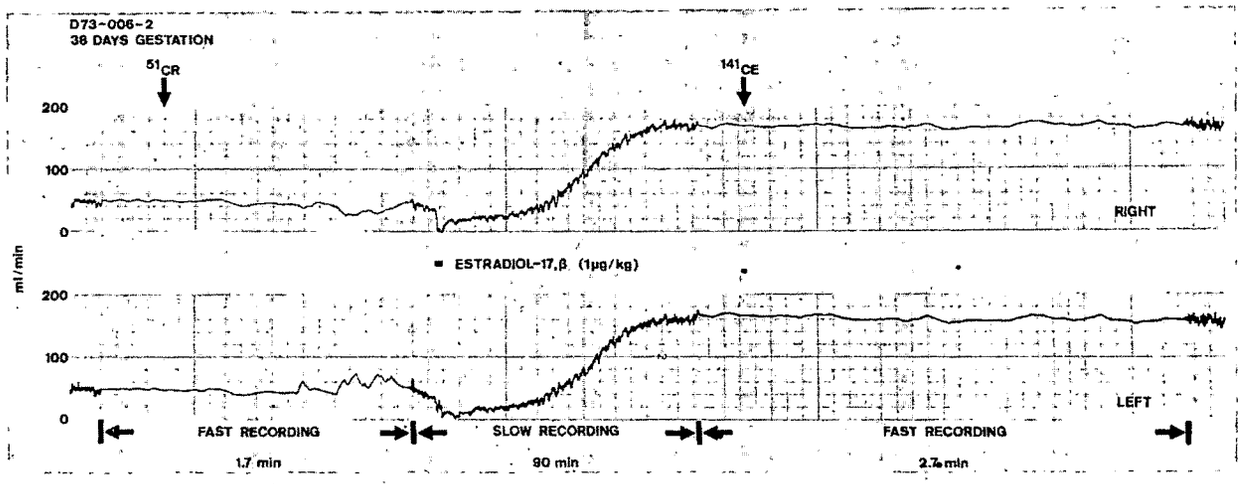


Fig. 1. Continous recording of blood flow in the right and left uterine arteries at 38 days of gestation in a sheep with twin pregnancy. Indicated are the times of the microsphere infusions and the two-minute intravenous infusion of estradiol.

operatively, the animals were maintained in large restraining pens, and uterine blood flow was measured daily with square-wave electromagnetic flow meters.* Venous and arterial pressures were measured simultaneously with the uterine blood flow. The flow and pressure signals were electronically integrated and recorded.† When the animals were fully recovered from the operation, usually within five to seven days, and while they remained standing in their stalls, radionuclide-labeled microspheres were infused into the left ventricle before the intravenous injection of 1 μg per kilogram of E_2 and at the time of maximum uterine vasodilation as evidenced by continuous flow meter monitoring (Fig. 1). The injection of the hormone lasted two minutes. The maximum vasodilation occurred approximately 100 minutes after the injection.

In order to assess the effect of estradiol on uterine oxygen consumption, arterial and uterine venous blood samples for O_2 determinations were drawn from seven of the pregnant ewes immediately before the administration of the hormone and again when the maximum vasodilatory effect of E_2 was observed. Uterine oxygen consumption was determined as the product of total uterine blood flow times the uterine arteriovenous oxygen content difference.

Surgical procedures. All food was withheld from the animals 48 hours prior to surgery, with ad libitum access to water. On the day of operation they were sedated with intravenous pentobarbital (5 mg. per kilogram), and a radiopaque catheter (1.2 mm. inside diameter by 2.2 mm. outside diameter) was passed into the left ventricle as previously described.[13] The sheep were then given spinal anesthesia (6 mg. of tetracaine hydrochloride) supplemented with intravenous pentobarbital as required. Through a low midline incision, the nonpregnant uterus was identified; bilateral oophorectomy was performed, and flow probes* were placed around both uterine arteries as described by Killam and associates.[5] In the pregnant ewes the uterine arteries were located within the broad ligament, and right-angle flow probes were placed on each main artery just proximal to the first branching. The major uterine vein on each side of the uterus was then catheterized.[8] The uterine vein catheters and leads from the flow probes were brought out of the abdomen through a single wound and secured at that point with sutures. Polyvinyl catheters (0.9 mm. inside diameter, 1.2 mm. outside diameter) were passed into both femoral arteries to a level just below the trifurcation of the abdominal aorta. Another catheter of the same size was placed in the inferior vena cava to the level of the diaphragm via a femoral vein. All catheters and flow probe leads were carried to the flank through subcutaneous tunnels and placed into an external pouch. The catheters were flushed daily with heparinized saline, (1,000 U. per milliliter). Penicillin (600,000 U.) and streptomycin (0.5 Gm.) were given on the day of operation and on the subsequent three days.

*Micron Instruments, Inc., 1519 Pontius Ave., Los Angeles, California 90025.

†Model 220, Brush recorders, Gould, Inc., 18901 Euclid Ave., Cleveland, Ohio 44117.

*Micron Instrument Co.

Table I. Total number of microspheres and disintegrations per minute (DPM) of radioactivity infused.

Isotope	*No. of microspheres infused*	*DPM infused*
^{141}Ce		
Mean	804,000	10,310,000
±S.D.	159,000	5,950,000
±S.E.M.	35,000	1,330,000
^{51}Cr		
Mean	1,695,000	12,350,000
±S.D.	578,000	6,520,000
±S.E.M.	129,000	1,460,000

Table II. The per cent of the mean arterial DPM in the uterine vein and high inferior vena cava before and after estradiol-17β

	Per cent of mean arterial DPM	
	Before	*After*
Inferior vena cava		
Mean	2.23	1.60*
±S.E.M.	0.68	0.55
No.	16	16
Uterine vein		
Mean	0.229	0.436†
±S.E.M.	0.053	0.144
No.	13	13

*The difference is not significant at $P > 0.20$.
†The difference is not significant at $P > 0.10$.

Microsphere infusion. Radioactive microspheres* with a specific activity of 10 mCi. per gram and a mean diameter of 25 $\mu \pm 5$ S.D. were employed. Approximately 150 μCi of ^{141}Ce and 350 μCi of ^{51}Cr were administered to each animal. The mean disintegrations per minute (DPM) and number of microspheres infused with each isotope are presented in Table I. The methods of infusing the microspheres and determining the number infused have been described.[13] Prior to the microsphere infusion, reference samples were withdrawn from both femoral arterial catheters into counting vials by a pump† at a rate of 3.88 ml. per minute for three minutes. Simultaneous samples were also withdrawn from one of the uterine vein catheters and the femoral vein catheter for an equal period of time in order to detect the escape of microspheres into the venous circulation from the uterine vascular bed and other vascular beds in the body. The DPM in these samples were compared to the DPM in the arterial sample (Table II). There was no significant loss of microspheres from the uterine vascular bed either before or after the vasodilatory effects of E_2, confirming the results of previous studies from this laboratory.[7, 14] There was a significant shunting of microspheres across other vascular beds into the inferior vena cava, equaling approximately 2 per cent of the infused number of microspheres. This figure did not change significantly following an infusion of E_2.

Tissue preparation. The ewes were weighed and killed within two to four hours after the second microsphere infusion and dissected for tissue preparation. All tissues were placed in preweighed, numbered counting vials (25 mm. in diameter, 80 mm. in length). The preparation of the nonpregnant and pregnant uteri and other tissues was as previously described.[14] Samples of skeletal muscle were taken from the lateral chest wall and hind limb. Skin samples were taken from the foreleg, chest, and the skin overlying the mammary gland.

Radioactive assay. The samples were counted in an automatic dual-channel gamma counter* with a three-inch crystal detector and a wide well (1⅛ inches in diameter), by a method previously reported.[13]

Oxygen assay. Whole-blood oxygen content was measured with a gas chromatograph† equipped with a blood gas accessory.[9]

Date reduction. Individual organ blood flows were calculated with the integrated arterial reference samples[6]:

$$\text{Organ blood flow (ml./min.)} = \frac{\text{total DPM in an organ}}{\text{mean DPM in arterial samples}} \times \text{rate of withdrawal.} \quad 1.$$

With a similar equation, cardiac output was determined simultaneously:

$$\text{Cardiac output (ml./min.)} = \frac{\text{total DPM infused}}{\text{mean DPM in arterial samples}} \times \text{rate of withdrawal.} \quad 2.$$

For the purpose of statistical analysis the animals are

*Minnesota Mining and Manufacturing Co., St. Paul, Minnesota 55101.

†Harvard Apparatus Co., 150 Dover Rd., Millis, Massachusetts 02054.

*Nuclear-Chicago Corp., 2000 Nuclear Dr., Des Plaines, Illinois 60018.

†GC-2A, Beckman Instruments, Inc., 2500 Harbor Blvd., Fullerton, California 92634.

Table III. Blood flow to the uterine tissues of nonpregnant and pregnant ewes, before and 100 minutes after the injection of 1 μg per kilogram of E_2. The hormone was injected intravenously over two minutes

Tissue	*Blood flow (ml./min.)*	
	Before E_2	*After E_2*
Total uterine blood flow		
Nonpregnant	23.36 ± 2.87	346.20 ± 28.27*
Group I	358.90 ± 69.28	625.40 ± 76.63*
Group II	1,099.00 ± 173.50	1,575.00 ± 231.70*
Myometrium		
Nonpregnant	9.46 ± 1.73	148.50 ± 23.11*
Group I	25.20 ± 4.92	113.70 ± 22.21*
Group II	35.28 ± 5.10	122.70 ± 10.94*
Endometrium		
Nonpregnant	7.76 ± 1.27	110.10 ± 14.86*
Group I	116.00 ± 15.78	295.30 ± 41.33*
Group II	157.60 ± 22.97	314.90 ± 39.35*
Cotyledons or Caruncles		
Nonpregnant	7.23 ± 1.26	87.59 ± 11.09*
Group I	214.70 ± 46.23	252.50 ± 42.81†
Group II	906.20 ± 155.30	1,138.00 ± 119.50†

The mean ± 1 S.E.M. is presented.

*The change in flow is significant at $P < 0.005$ (paired t test).

†The change in flow is significant at $P < 0.05$ (paired t test).

divided into three groups in the tables and figures: six nonpregnant ewes, seven ewes at 38 to 78 days of gestation (Group I), and six ewes at 120 and 139 days of gestation (Group II).

Results

Effect of E_2 on the uterine vascular bed. In previous reports we documented the inhibitory effect of surgical stress on the response of uterine blood flow to E_2 in nonpregnant ewes[5, 13] and thus performed this series of experiments after the animals had recovered from operation and had resumed a normal diet. In both pregnant and nonpregnant ewes there was a significant increase in uterine blood flow following the systemic infusion of 1 μg per kilogram of E_2 (Table III). The average increase in flow in milliliters per minute (Fig. 2, *C*) was not significantly different among the nonpregnant and the two pregnant groups and was equal to 351 ml. per minute ± 36 S.E.M. The same data expressed as a per cent change in flow (Fig. 2, *B*) demonstrate a mean increase of 1,455 per cent ± 197 S.E.M. in the nonpregnant ewes, while in the pregnant animals there was a gradual decline in the relative magnitude of the change from 225 per cent at 38 days to a mean of 43 per cent near term (Group II).

As shown in Table III and Figs. 3, 4, and 5, all three

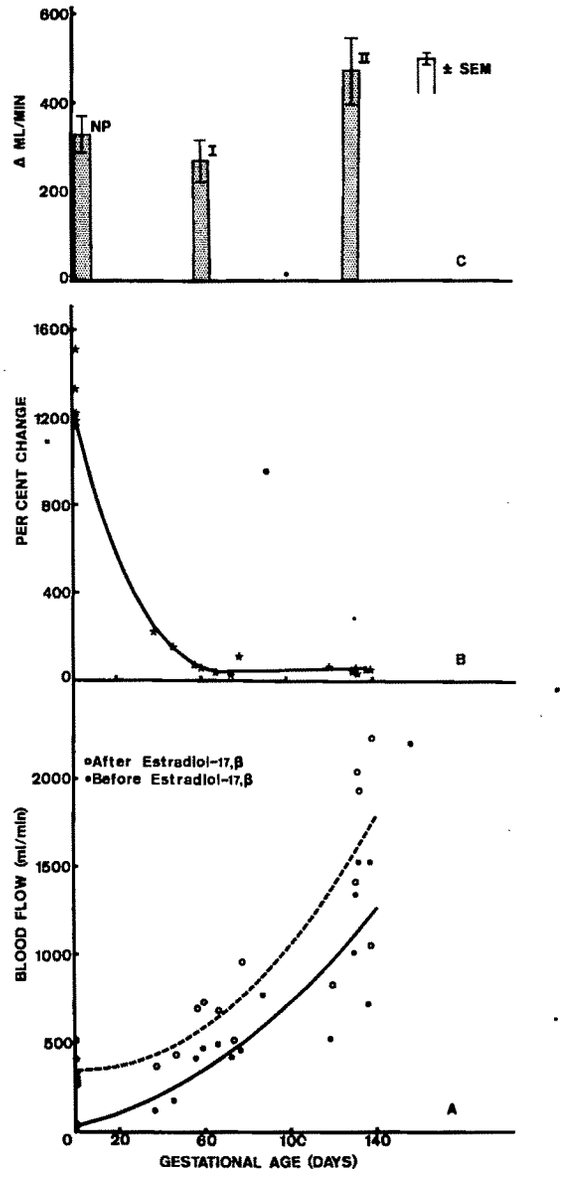


Fig. 2. Effect of estradiol-17β on total uterine blood flow. Graph *A* shows the flow before and after estradiol in milliliters per minute; *B*, the per cent change in flow; and *C*, the absolute change in flow (milliliters per minute) in nonpregnant, early pregnant (Group I), and late pregnant (Group II) ewes.

major uterine tissues, i.e., myometrium, endometrium, and cotyledons (represented by the caruncles in the nonpregnant uterus), showed significant increases in blood flow following an infusion of E_2, but the pattern and magnitude of response throughout gestation were different for each tissue. Myometrial blood flow increased after estrogen stimulation to approximately the same maximum (128 ml. per minute ± 11.4

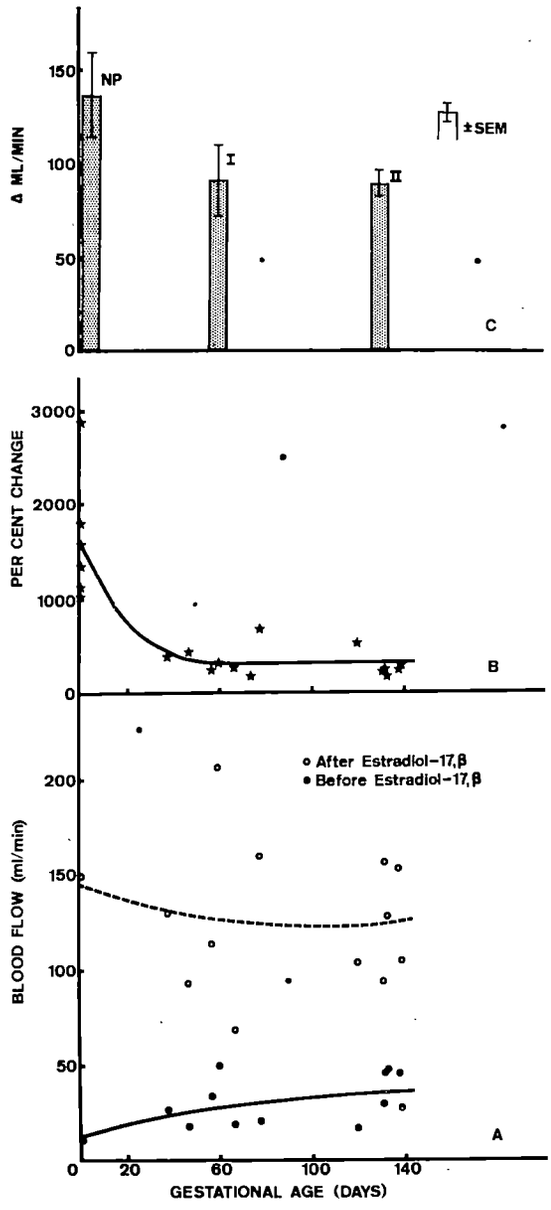


Fig. 3. Effect of estradiol-17β on myometrial blood flow. Graph *A* shows the flow before and after estradiol in milliliters per minute; *B*, the per cent change in flow; and *C*, the absolute change in flow (milliliters per minute) in nonpregnant, early pregnant (Group I), and late pregnant (Group II) ewes.

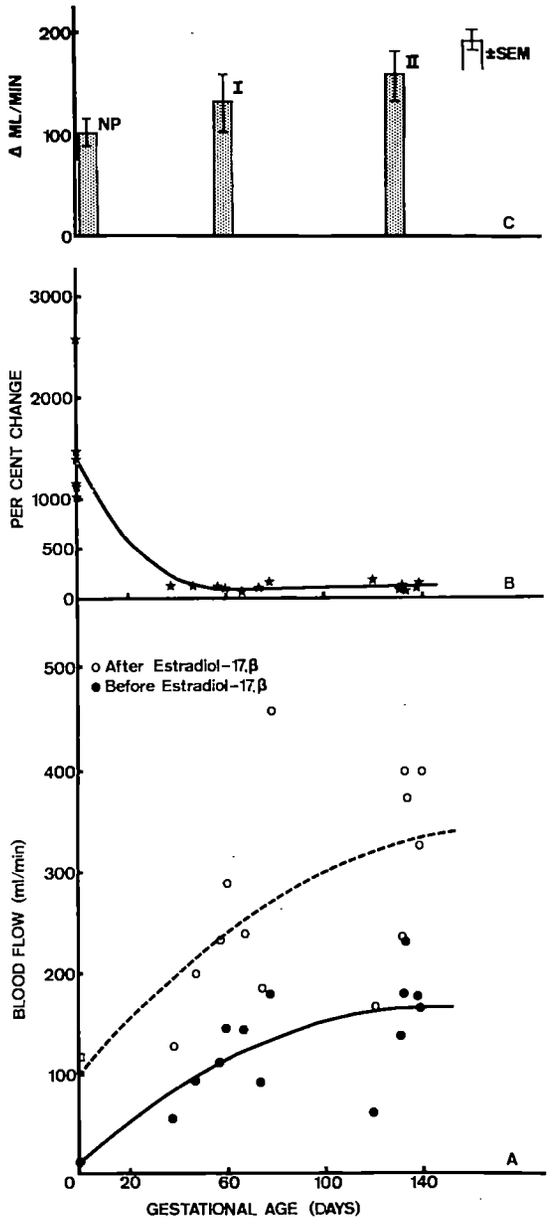


Fig. 4. Effect of estradiol-17β on endometrial blood flow. Graph *A* shows the flow before and after estradiol in milliliters per minute; *B*, the per cent change in flow; and *C*, the absolute change in flow (milliliters per minute), in nonpregnant, early pregnant (Group I), and late pregnant (Group II) ewes.

S.E.M.) in pregnant and nonpregnant ewes despite a significant increase of the base-line flow during gestation. On the contrary, both endometrial blood flow and estrogen-stimulated endometrial blood flow did increase during gestation (Fig. 4, *A*). In late pregnancy the response of the placental blood flow to E_2 stimulation was not as pronounced as that of myometrial and endometrial flows. In the group at 120 to 139 days (Group II), the average increase of placental blood flow was 25 per cent, whereas endometrial flow doubled and myometrial flow increased 3.5-fold. Since E_2 stimulation causes an enormous increase of blood flow to the sites of implantation (caruncles) in the nonpregnant animal,[13] it is apparent that at some time in pregnancy there is a decrease in the vasodilatory effect of exogenous estrogen on the placental ar-

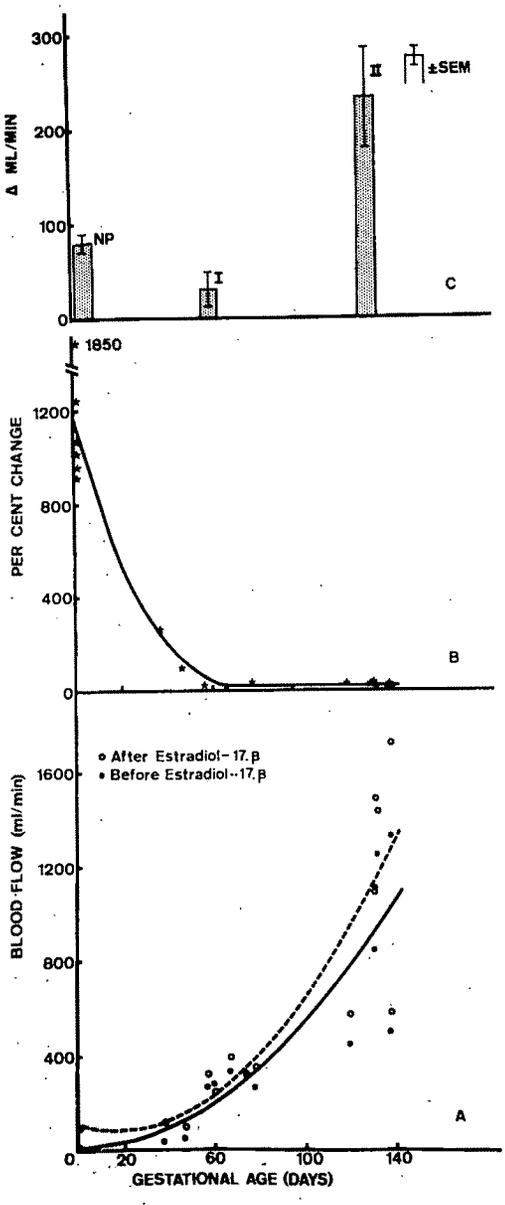


Fig. 5. Effect of estradiol-17β on blood flow on the placental cotyledons. *A* is the flow before and after estradiol in milliliters per minute; *B* is the per cent change in flow, and *C* is the absolute change in flow (milliliters per minute), in the caruncles of the nonpregnant ewe and in the cotyledons of early (Group I) and late (Group II) pregnancy.

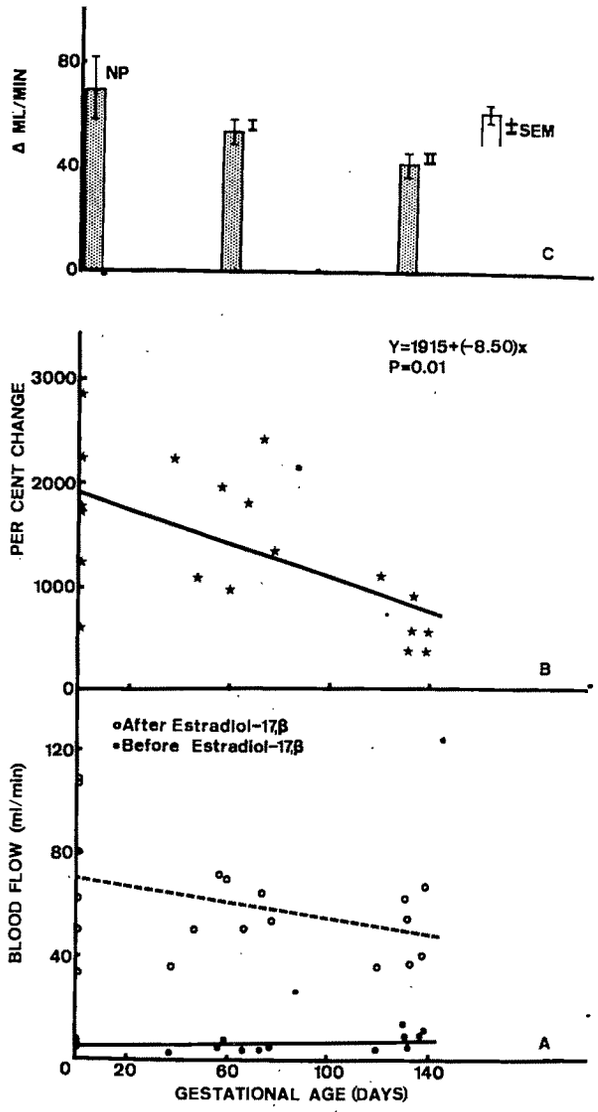


Fig. 6. Effect of estradiol-17β on cervical blood flow. *A* is the flow before and after estradiol in milliliters per minute; *B* is the per cent change in flow, and *C* is the absolute change in flow (milliliters per minute) in the nonpregnant, early pregnant (Group I), and late pregnant (Group II) ewe.

terioles. This time can be located at approximately the second month of gestation by considering the experiments within Group I which extended from the thirty-eighth to the seventy-eighth day of pregnancy. At 38 days, estrogen stimulation caused a marked increase of cotyledonary flow from 30.6 to 109.5 ml. per minute, i.e., a 2.6-fold increase. In the experiment at 47 days, E_2 caused the cotyledonary flow to double, from 45.1 to 89.7 ml. per minute. In the next two experiments at 60 days, the effect of exogenous E_2 on cotyledonary blood flow was virtually zero, with a mean increase from 265.2 to 272.8 ml. per minute. In the next group of three experiments performed between the sixty-seventh and seventy-eighth days, there was an average increase of 13 per cent, from 299.0 to 340.9 ml. per minute.

Effect of E_2 on other reproductive tissues. There was a significant vasodilatory effect of E_2 on blood flow to the tissues of the cervix, vagina, ovaries, uterine tubes, and mammary gland throughout pregnancy

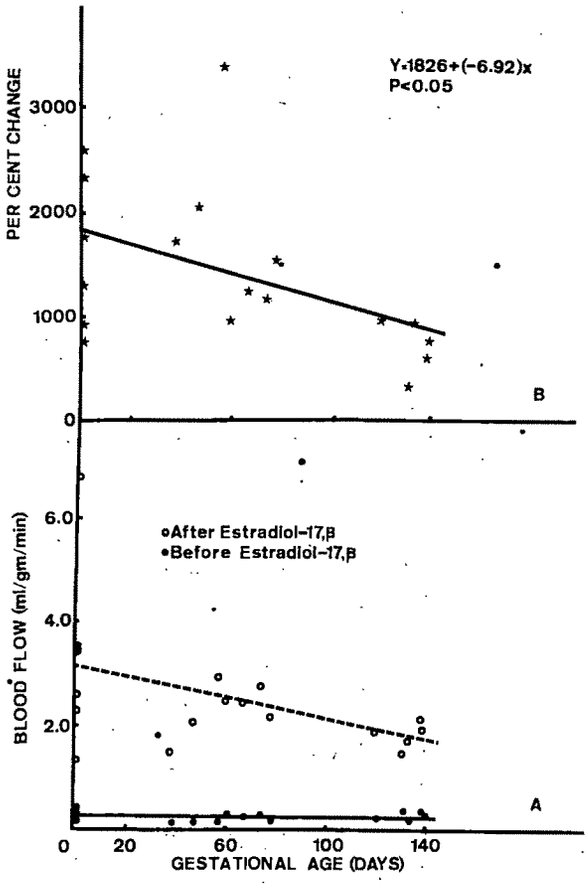


Fig. 7. Effect of estradiol-17β on vaginal blood flow plotted against gestational age. *A* is the flow before and after estradiol in milliliters per minute · gram, and *B* is the per cent change in flow.

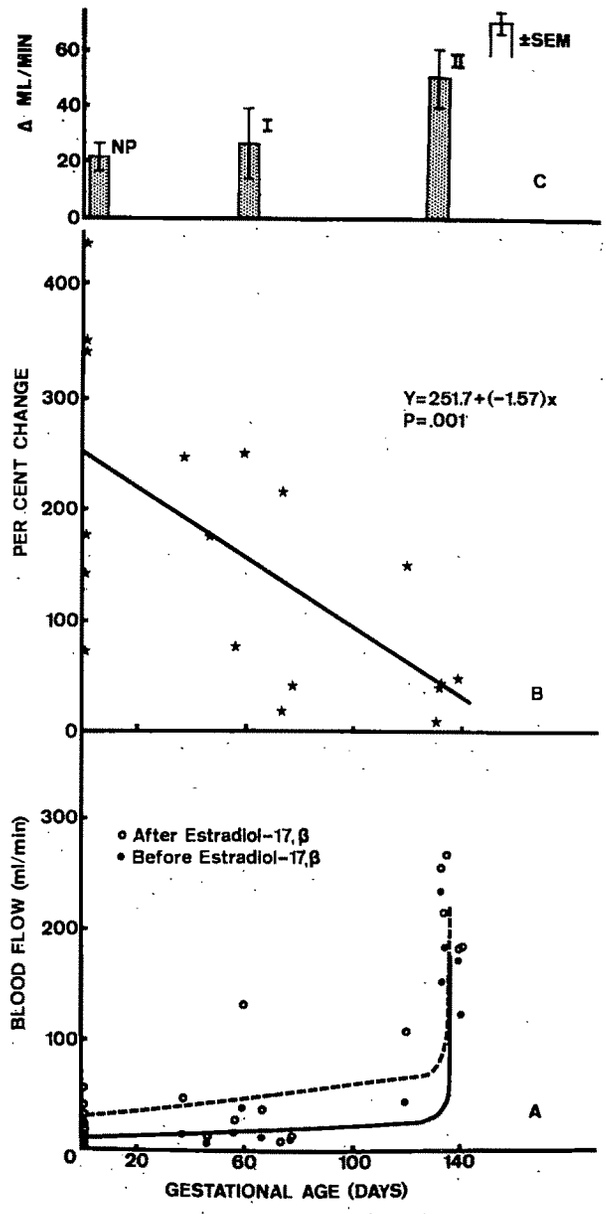


Fig. 8. Effect of estradiol-17β on blood flow to the mammary gland. *A* is the flow before and after estradiol in milliliters per minute; *B* is the per cent change in flow, and *C* is the absolute change in flow (milliliters per minute) in the nonpregnant, early pregnant (Group I), and late pregnant (Group II) ewe.

(Table IV). As in the uterine tissues, as pregnancy progressed toward term, the cervix (Fig. 6), vagina (Fig. 7), and mammary gland (Fig. 8) demonstrated a reduction in the per cent change in flow following an infusion of E_2. This phenomenon was not evident in the uterine tubes and ovaries.

Effect of E_2 on cardiac output. The reliability of simultaneous measurements of cardiac output and tissue or organ blood flows with the microsphere method has been reported.[15] This method was employed in these experiments to demonstrate the effect of E_2 on cardiac output in four of the nonpregnant animals and all of the pregnant animals included in this study. There was a mean increase in cardiac output of 13.3 ml. per minute · kilogram ± 4.25 S.E.M. ($P < 0.005$) at 60 to 90 minutes following an infusion of E_2. This is an increase of 14.5 per cent ± 4.51 S.E.M. A similar increase was observed by Parer and associates[10] following the infusion of large doses of estrone. No significant change in mean arterial pressure was noted: 97.9 mm. Hg before E_2 and 96.8 mm. Hg afterward ($P > 0.40$). There was a significant change in heart rate, which increased from 100 to 111 beats per minute ($P < 0.001$). In the last animal studied, the E_2 was administered in isotonic saline, 1 μg per milliliter, rather than in the carrier solution previously employed, which contains ethyl alcohol and

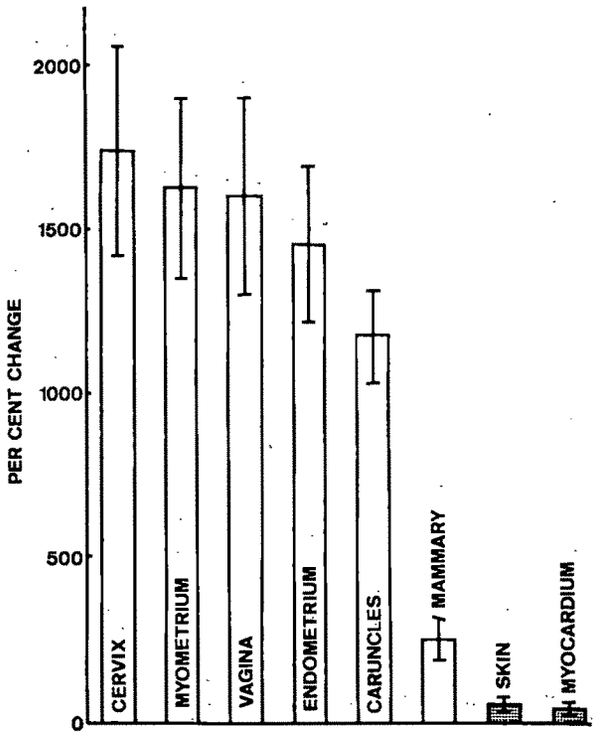


Fig. 9. Comparison of the per cent change in those tissues in the nonpregnant ewe which demonstrated a significant change in flow ($P < 0.01$) following a systemic infusion of estradiol-17β (1 μg per kilogram). The nonreproductive tissues are represented by the shaded bars. The means ± 1 S.E.M. are presented.

Table IV. The effect of estradiol-17β (E_2) on blood flow to reproductive tissues of pregnant and nonpregnant ewes. Same experimental conditions as Table III

Tissue	*Blood flow*	
	Before E_2	*After E_2*
Cervix (ml./min.)		
Cervic (ml./min.)		
Nonpregnant	4.15 ± 0.45	73.38 ± 12.45*
Group I	3.57 ± 0.62	56.63 ± 4.86*
Group II	8.01 ± 1.62	49.94 ± 5.06*
Vagina (ml./min · Gm.)		
Nonpregnant	0.207 ± 0.042	3.31 ± 0.77†
Groups I and II	0.185 ± 0.024	2.13 ± 0.14*
Uterine tubes (ml./min.)		
Groups I and II	0.474 ± 0.086	4.01 ± 0.28*
Ovaries with corpus luteum (ml./min.)		
Group I	9.12 ± 1.14	14.81 ± 1.29*
Group II	4.82 ± 0.51	8.20 ± 1.11†
Ovaries without corpus luteum (ml./min.)		
Group I and II	0.252 ± 0.029	1.00 ± 0.24†
Mammary (ml./min.)		
Nonpregnant	10.58 ± 2.79	32.06 ± 5.58†
Group I	15.75 ± 3.51	39.74 ± 16.21†
Group II	151.70 ± 26.34	200.70 ± 23.49†

The pregnant animals are divided into Groups I and II unless there is no significant difference between groups. The mean ± 1 S.E.M. is presented.

*The change in flow is significant at $P < 0.005$.

†The change in flow is significant at $P < 0.05$.

propylene glycol.[5] The increase in cardiac output in this ewe was much greater than that noted in the other animals studied, 67.4 per cent or 67.2 ml. per minute · kilogram, and suggested the possibility of a depressant effect on cardiac output by either of the nonestrogenic compounds in the carrier solution. This possibility was investigated in nonpregnant oophorectomized goats equipped with an electromagnetic flow probe around the ascending aorta.*

The carrier solution was found to depress cardiac output 8.2 per cent approximately two hours after administration, whereas saline alone had no effect. Injection of E_2 dissolved in saline led to a 16.3 per cent increase of cardiac output at two hours in one goat. Thus, it appears that we might have documented greater increases in cardiac output following E_2 administration if saline had been used as the solvent.

Effect of E_2 on nonpregnant tissues. In addition to the vascular response of the reproductive tissues to a systemic infusion of E_2, the nonreproductive tissues were also investigated. In the nonpregnant group of ewes (Fig. 9), only the skin and myocardium demonstrated a significant change in flow following E_2 ($P < 0.025$). Those tissues in the nonpregnant ewes which were examined but did not demonstrate a significant response to E_2 included the kidneys, adrenals, spleen, brain, pancreas, skeletal muscle, and fat. In the pregnant ewes (Fig. 10), significant vasodilation occurred in the skin, adrenal glands, myocardium, and liver. The liver was the only nonreproductive tissue to demonstrate a change in arterial blood flow similar to that noted in the reproductive tissues, a mean increase of 457 per cent ± 262 S.E.M. ($P < 0.05$). The skeletal muscle in the pregnant ewes was the only tissue with a decrease in flow: −12.10 per cent ± 7.08 S.E.M. ($P < 0.05$). In the last three pregnant ewes studied, the vascular response of the thyroid gland was examined. There was a mean increase in flow of 137 per cent ± 34.7 S.E.M., but because of the small number studied this figure is of borderline significance.

Uterine oxygen consumption following E_2. Results

*The animals were prepared by Dr. C. Tucker for other experiments and were loaned to us for these observations.

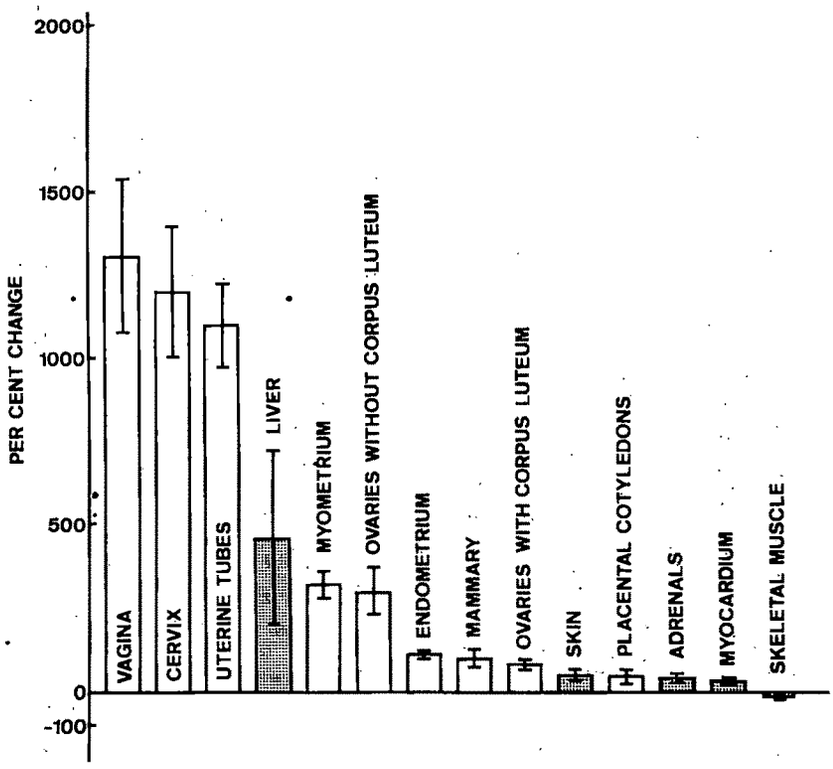


Fig. 10. Comparison of the per cent change in those tissues in the pregnant ewe which demonstrated a significant change in flow ($P < 0.05$) following a systemic infusion of estradiol-17β (1 μg per kilogram). The nonreproductive tissues are represented by the shaded bars. The means + 1 S.E.M. are presented.

Table V. Uterine oxygen consumption before and after a systemic infusion of estradiol-17β (E_2). Same experimental conditions as in Table III

Sheep No.	*Uterine blood flow (ml./min.)*		*A-V O_2 (mM.)*		*Oxygen consumption (ml./min.)*	
	Before	*After*	*Before*	*After*	*Before*	*After*
1	423	846	1.16	0.47	11.00	8.93
2	817	1,192	1.42	0.96	25.90	25.61
3	838	994	1.38	1.12	25.87	24.93
4	1,360	1,711	1.22	0.80	37.04	30.62
5	739	912	1.39	1.29	23.08	26.25
6	1,881	2,227	1.53	1.20	64.34	59.97
7	561	933	0.93	0.72	11.67	15.09
Mean	946	1,259	1.29	0.94	28.41	27.34
P*	<0.005			<0.005	N.S.	

A-V = Arteriovenous. N.S. = Not significant.
*One-tailed paired t test.

of the measurement of uterine oxygen consumption in seven of the pregnant ewes are presented in Table V. In each animal there was a significant increase in uterine blood flow following an infusion of E_2 and a concomitant decrease in uterine arteriovenous oxygen content difference. As a result, there was no significant change in uterine oxygen consumption despite the increase in uterine blood flow.

Comment

As shown in Table III and in agreement with results of previous studies,[13] the uterine vasculature of nonpregnant, oophorectomized ewes is extremely sensitive to the vasodilatory effect of physiologic doses of E_2, exhibiting a fifteen-fold increase in flow. This increase in flow is approximately the same for each of the individual uterine tissues, endometrium, myometrium, and caruncles. It should be noted that the arterioles supplying blood to the caruncles are the same arterioles supplying blood to the placental cotyledons. Contrary to this observation in nonpregnant animals, Greiss and Marston[3] have demonstrated an increase in uterine blood flow after estrogen stimulation of only 45 per cent in pregnant ewes in the last third of gestation. This suggests that the pregnant state alters the ability of at least some parts of the uterine vascular bed to vasodilate in response to exogenous estrogen. Because of the method employed, Greiss and Marston could not



Fig. 11. Vinyl acetate cast of the terminal uterine arterial tree in the pregnant ewe, demonstrating *(A)* cotyledonary arterioles and a major cotyledonary artery, *(B)* endometrial artery, and *(C)* myometrial arteries.

demonstrate the response of the individual uterine tissues and thus were unable to determine if this altered response to estrogen occurred in each of the tissues or was peculiar to any one of them. This is an important consideration in the face of the redistribution of uterine blood flow that occurs during pregnancy, with approximately 80 per cent going to the placental cotyledons near term.[7, 14] It was the purpose of this investigation to describe the changes in the vasodilatory response to estrogen that occur in uterine tissues during gestation.

From the data presented, it is evident that the vasodilatory response to an infusion of exogenous estrogens is retained by each of the uterine vascular beds throughout pregnancy, but the response of these beds to estrogen is quite different from the proportional increase in flow which occurs in the nonpregnant state. In the last third of gestation, the per cent change in flow to each uterine tissue following an infusion of E_2 is significantly different: 307 per cent in the myometrium, 108 per cent in the endometrium, and 25 per cent in the placental cotyledons. This pattern of selective vasodilation by E_2 suggests that the site of vasodilation is beyond a common artery and most likely in the distal arterioles. However, a combination of effects on a common artery and the distal arterioles cannot be excluded. Fig. 11 shows the cast of a uterine artery in late pregnancy with placental *(A)*, endometrial *(B)*, and myometrial *(C)* branches. It is evident that each of these vessels has a separate point of departure from the common artery.

If the increase in flows following an infusion of estrogens in the nonpregnant, unstressed ewe represents maximum uterine vasodilation, then some inferences can be drawn from the data in this study as to the mechanisms responsible for the changes in blood flow to uterine tissues during pregnancy. In the myometrium there is a maximum level of vasodilation following an infusion of E_2 which is the same in nonpregnant and pregnant ewes and is greater than the increase in blood flow to that tissue during pregnancy (Fig. 3, *A*). This suggests that the increase in myometrial blood flow during pregnancy may be secondary to partial vasodilation of existing vasculature, with little or no growth of new myometrial vessels. It also demonstrates that in the myometrium the vasodilatory response to exogenous estrogen is not significantly altered during gestation. In the endometrium and placental cotyledons, the findings are quite different. The blood flows to the nonpregnant endometrium and caruncles following an infusion of E_2, 110 and 87.6 ml. per minute, respectively, are lower than the levels of blood flow reached in either the endometrium or placental cotyledons near term, 158 and 906 ml. per minute, respectively. Furthermore, the blood flow after estrogen stimulation is much greater in the pregnant animal. The implication here is that the increase in blood flow during pregnancy to these two tissues is not explainable by a mechanism as simple as vasodilation of pre-existing vasculature. It would appear that, in addition to vasodilation, there are hypertrophy and/or a growth of vessels in the endometrium and placental cotyledons.

Another question which lends itself to speculation is why there exists a difference in the responses of the individual uterine tissues to the vasodilatory effects of E_2. In ovine pregnancy the blood levels of estrogen are relatively small[1] in comparison to those of human pregnancy.[11] However, the ovine placenta is capable of producing estrogens.[16] This phenomenon implies that there may be concentrations of estrogen in the

placental tissues which are greater than those noted in the circulating blood[1] and other uterine tissues. Therefore, the placenta may have a greater degree of ongoing vasodilation secondary to a local, higher concentration of estrogen as compared to endometrium and myometrium. This, in turn, might cause the placental response to exogenous E_2 to be less than that seen elsewhere in the uterus. A similar argument can be made for the different response to E_2 of ovaries with and without a corpus luteum. The per cent increase of blood flow in the ovaries with a corpus luteum in response to E_2 was 80 per cent, whereas it was 296 per cent in the ovaries without a corpus luteum (Table IV). The corpus luteum is an estrogen-producing tissue[16] whose high blood flow during gestation suggests that it may be partially vasodilated secondary to the effect of endogenous estrogens.

To the best of our knowledge, the present study is the first demonstration of the effect of estrogen on regional blood flow in nonanesthetized, unstressed animals and on the distribution of cardiac output. The significant vasodilatory response to estrogen documented in the cervix, vagina, uterine tubes, and mammary gland confirms results of an earlier study in nonpregnant ewes.[13] It is apparent that blood flow to nonreproductive tissues was rarely affected by estrogen, and those that were tended to respond minimally when compared with flows to reproductive tissues other than the placental cotyledons and ovaries with a corpus luteum (Figs. 9 and 10). The increase in myocardial blood flow can be explained by the increase in cardiac output and heart rate that follows an infusion of E_2. The vasodilation in the liver and adrenal glands may be the result of circulating metabolic products rather than the presence of local estrogen receptor sites as in the reproductive tissues. On the other hand, the response in the skin could be analogous to the hyperemia of estrus seen in the specialized sexual skin of lower primates, suggesting that there are, in fact, estrogen receptor sites in the vasculature of the skin.

As shown in Table V, there was no change in uterine oxygen consumption following the infusion of E_2. This observation extends the findings of Huckabee and colleagues,[4] who reported a similar lack of effect on oxygen consumption following estrogen stimulation of uterine blood flow in nonpregnant ewes and lends additional support to the concept that uterine blood flow is not controlled by the partial pressure of respiratory gases in the uterine circulation.

The results of the present study should not be construed as evidence in support of the concept[2] that estrogen administration to the mother may be used to improve fetal oxygenation. First, the effect of estrogens on increasing normal placental blood flow in late pregnancy is relatively modest. Second, estrogens may be ineffective in restoring to normal uterine blood flow which is abnormally low. In the two pregnant ewes that were not included in the results because of failure to recover after operation, uterine blood flow at the time of E_2 administration was approximately half of normal. In these animals the injection of E_2 caused only a 9 per cent increase of flow to the placental cotyledons. Previous studies from our laboratory have shown that acute stress, such as that associated with tying the animal to the operating table for one or more hours, curtails the effect of estrogens on the uterine blood flow of nonpregnant ewes.[5]

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Antibiotic treatment of parturient women colonized with group B streptococci

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A prospective study was conducted among third-trimester parturient women with cervical or urethral colonization with group B streptococci to determine the influence of antibiotic treatment on subsequent colonizations among their infants. Cultures were obtained from dry swabs inoculated directly onto selective blood agar media containing neomycin and naladixic acid. Seventy-four women were found to be colonized among 1,098 cultured (7 per cent). A significant reduction in colonization was noted among mothers treated with ampicillin within three weeks of completion of therapy. This difference was no longer apparent at delivery. There was likewise no difference in the colonization rate of infants in the treatment and no-treatment groups. The data suggest that additional measures must be undertaken to prevent maternal recolonization.

THE GROUP B STREPTOCOCCUS has emerged as a significant pathogen in neonatal sepsis and meningitis in recent years.[1–6] Two distinct clinical forms of the disease have been described.[4–7] An early form is characterized by rapid onset of respiratory distress, sepsis, and shock. A very high mortality rate is associated with this form of the disease. In nearly all reported cases, the source of colonization of the infant has been the birth canal of the pregnant mother.[4, 7–10] Serologic subtyping of the group B streptococci has been identical in infant and maternal vaginal cultures.[7, 9, 10]

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Late-onset disease characteristically occurs between three and six weeks and has been termed a "meningitis form."[4, 11] The vast majority of reported cases have been associated with meningitis; however, mortality rates have been considerably lower than in the early-onset diseases.[6, 7] The source of colonization of these infants has not been determined.

Franciosi and associates[4] describe the early-onset disease as being associated primarily with B-I subtypes, although Baker and colleagues[7] have found Type III to be responsible for 75 per cent of early-onset disease in their experience. Late-onset disease has been primarily associated with Type III strains.[4, 7–9]

There are no convincing data which indicate that colonization rates of pregnant women have increased in recent years. Hood and co-workers[1] reported a colonization rate of 5 per cent from New Orleans, in 1961, which is similar to the incidence in the parturient population from the Denver area reported in 1973.[4] Notable exceptions are the 23 per cent vaginal colonization rate reported by Baker and Barrett[10] using a selective antibiotic broth media and a 29 per cent colonization rate of third-trimester women noted in Palm Beach County, Florida, where multiple cultures

Table I. Effect of ampicillin treatment on maternal and infant colonization with group B streptococci

	Treatment (27)		*No treatment (34)*		
	Positive	*Negative*	*Positive*	*Negative*	*Significance*
Mothers, follow-up 3 wk.	4	23	14	20	$\chi^2 = 3.84$; $p = 0.05$
Mothers, at delivery	8	15 (4 unknown)	13	16 (5 unknown)	N.S.
Infants, at delivery	6	21	10	24	N.S

N.S. = Not significant.

were obtained.[12] The high colonization rates in the latter two studies may be a reflection of different methods used for isolation and identification of group B streptococci.

Based upon an attack rate of two to three per 1,000 parturient women, whose infants had a very high mortality rate, Franciosi and associates have suggested the antepartum treatment of pregnant women harboring vaginal group B streptococci in an attempt to eradicate the organism before delivery and prevent colonization of infants.[4] Successful eradication was demonstrated in 13 of 14 pregnant women treated with penicillin in an initial preliminary study. This approach is supported by McCracken[11] who feels that pregnant women with proved colonization should be treated routinely. This point of view is not shared by Baker and Barrett[10] who feel that the high incidence of colonization among parturients with a relatively low attack rate makes antibiotic prophylaxis of gravid female carriers impractical. Eickhoff and associates[13] have expressed similar disagreement with the routine treatment of pregnant women because of lack of data which support the successful eradication of the streptococcal flora from the birth canal and subsequent colonization of their infants and the potential risk of adverse reactions to antibiotic treatment.

In an attempt to determine the efficacy of antibiotic treatment of group B streptococcal colonization of parturient women and their infants, a prospective study was undertaken at the Kansas City General Hospital.

Patients and methods

Maternal cervical and urethral cultures were obtained in the third trimester of pregnancy from sterile dry swabs inoculated directly onto selective blood agar media containing naladixic acid (15 mcg. per milliliter) and neomycin (30 mcg. per milliliter). Identification of group B streptococci was made by colony morphology, a typical zone of hemolysis, resistance to bacitracin, and the ability of the organism to hydrolyze hippurate.[14] Subtyping was performed with the slide agglutination method of Cropp and colleagues.[15] Antisera were supplied by the Streptococcal Disease Section of the Center for Disease Control in Fort Collins, Colorado. Test strains were obtained from Dr. Rebecca Lancefield.

Third-trimester pregnant women were divided into two groups by random assignment dictated by the last number of the chart. Treatment mothers received oral ampicillin, 500 mg. four times daily for seven days. The control group received no medication. Repeat cultures were obtained of the cervix and urethra at the end of antibiotic therapy in the treatment group and at the same time in the control group. No attempt was made to determine the compliance of the treatment group to ensure adherence to the antibiotic regimen. Attempts were made to obtain urethral cultures of the male sexual partner; however, because of a lack of identification and compliance of these partners, this was not possible, and this phase of the study was abandoned.

Repeat cervical and urethral cultures were obtained in both groups at the time of delivery. Immediately following admission of the infant to the nursery, cultures were obtained of the infants' nares, oropharynx, umbilicus, ear canal, and rectum. These culture swabs were processed in the same way as maternal cultures. Antibiotic therapy of infants was dictated by the clinical course and was not controlled by the results of infant culture data. Blood, urine, and cerebrospinal fluid cultures were obtained prior to antibiotic therapy when utilized. Data collection sheets were prepared which gave detailed pertinent maternal and infant clinical data.

Results

In the third trimester of pregnancy, 1,098 women were cultured and 74 patients (7 per cent) with cervical and/or urethral colonization were identified. Sixteen patients had positive urethral cultures alone. Five had positive cervical cultures alone, and the remainder were culture-positive in both sites.

Sixty-one patients returned within three weeks of completing the treatment or no-treatment regimen and constitute the study population. Culture results are shown in Table I. The initial follow-up cultures

Table II. Infant colonization with Group B streptococci in relation to maternal colonization at delivery

Maternal colonization	*Treatment (27)*			*Maternal colonization*	*No treatment (34)*		
	Positive	*Negative*	*Not done*		*Positive*	*Negative*	*Not done*
8 Positive	5	3	0	13 Positive	6	7	0
15 Negative	1	13	1	16 Negative	4	12	0
4 Unknown	0	3	1	5 Unknown	0	4	1 (Stillborn)
27	6	19	2	34	10	23	1

revealed a significant difference between the treatment and no-treatment groups. Of the 27 treated with ampicillin, cultures of four patients remained positive, whereas 14 of the 34 patients who received no treatment had positive cultures. However, at the time of delivery, this difference was no longer apparent. There were eight positive, fifteen negative, and four unknown cultures in the treatment group. There were 13 positive, 16 negative, and five unknown cultures in the no-treatment group. There was likewise no difference in colonization of infants in the two groups at the time of delivery.

Infant culture data suggest that multiple sites should be obtained since the umbilicus was the only positive culture in two infants and the nose or throat alone in two infants. Multiple sites were colonized in the remaining 12 patients.

Table II depicts the group B streptococci flora of infants in relation to their mothers' cultures. In the treatment group, five of the six culture-positive infants had mothers whose cultures were positive. In the no treatment group, six of the 11 culture-positive infants had culture-positive mothers. The five infants born to culture-negative women probably reflect an inadequate identification technique of positive maternal colonization.

It is interesting to note that seven infants born to mothers who initially had positive cultures, but whose cultures were unknown at the time of delivery, had negative cultures. This could readily be explained by chance alone; however, negative cultures were also obtained from 11 of the 13 mothers who were initially known to be colonized with group B streptococci but who failed to have follow-up cultures during pregnancy and were deleted from the study group. Over all, the entire group of 18 infants born to mothers who were initially colonized in the third trimester of pregnancy but whose colonization status at the time of delivery was unknown had negative cultures of multiple sites at birth. We have no explanation for this phenomenon.

Serologic subtypes of group B streptococci are shown in Table III. There was 100 per cent correlation between maternal and infant subtypes. Type B-Ic reactions were agglutinated by Ia antisera and reported as Type Ia. Approximately 30 per cent of the strains which could be typed were Type III which is consistent with the experience of Franciosi and associates,[4] Baker and Barrett,[10] and Wilkinson and colleagues.[9] Three maternal and two infant cultures agglutinated both Ib and II antisera and probably represent mixed flora rather than true cross-reactions. No cross-reactions occurred with test strains. There were very few strains which could not be typed. There were too few infants with clinical disease to correlate the occurrence of illness with the serotype.

Table III. Subtypes of group B streptococci

Subtype	*Mothers (61)*	*Infants (16)*
Ia	6	3
Ib	13 } 3 Mixed	3 } 2 Mixed
II	16 }	6 }
III	20	4
Unknown	9 (1*)	2 (1*)
	64	18

*Could not be typed.

Table IV depicts clinical information regarding the perinatal period. The mean birth weight and gestational age were consistent with those of term infants, and only two were born prematurely. There was a paucity of perinatal complications. Three infants in the no-treatment group had premature rupture of the membranes more than one hour prior to the onset of labor; however, none had prolonged rupture of membranes. None of the sixteen infants had abnormally low Apgar scores. One infant from each group developed radiographic and clinical evidence of pneumonia. The serotypes of streptococci in these two infants were Ia and Ib, respectively. Both were treated with systemic penicillin and gentamicin and both survived. Blood cultures were obtained from 14 infants and all were negative. Seven infants in addition to the two with pneumonia received antibiotics primarily

Table IV. Clinical data associated with group B streptococcal colonization

	Treatment (6)	*No treatment (10)*
Birth weight	3248 Gm. ± 328 (S.D.)	3014 Gm. ± 543 (S.D.)
Gestational age	39.1 wk. ± 1.3 (S.D.)	39.1 wk. ± 2.0 (S.D.)
Duration of rupture of membranes (hr.)	0 – 4	0 – 15
Premature rupture of membranes	None	3
Perinatal asphyxia	None	None
>5 Polymorphonuclear leukocytes in gastric aspirate	3/5	2/6
Pneumonia	1	1
Antibiotic therapy	3	6
Sepsis or meningitis	None	None

because of the knowledge of the maternal colonization and concern on the part of the house officer of the potential danger of sepsis. No statement can be made regarding the routine treatment of culture-positive infants from the data in this study. Maternal complications were limited to one case of postpartum endometritis for which antibiotics were administered and two antepartum urinary tract infections from which group B streptococci were isolated from mixed flora of "clean-voided" specimens.

The data from the present study indicate that antibiotic therapy with oral ampicillin in the third trimester of pregnancy cannot be utilized by the methods employed to decrease maternal colonization of the genitourinary tract or the infant colonization rate at the time of birth. However, the data do indicate that a significant reduction in cervical and urethral colonization can be obtained within three weeks of completion of therapy. This suggests that recolonization occurs, probably through venereal transmission or through re-emergence of an undetectably low population of streptococci following antibiotic treatment.

These data should not be construed as evidence that antibiotic treatment has no place in the management of the pregnant woman. Rather it points out the difficulties encountered with such a regimen. It would appear that antibiotic therapy must be continued until the time of delivery and that further study of the male sexual partner is essential. Unfortunately, the latter problem was not possible in this study.

It is encouraging to note that 11 of the 16 colonized infants were delivered of mothers who were shown to be culture positive. The five infants delivered of mothers with negative cultures probably represent a failure of the maternal culture technique. Since completion of this study, there are published data available which demonstrate that utilization of broth media and repeated cultures will significantly improve the culture yield and reduce false negative results to a minimum.[10, 12]

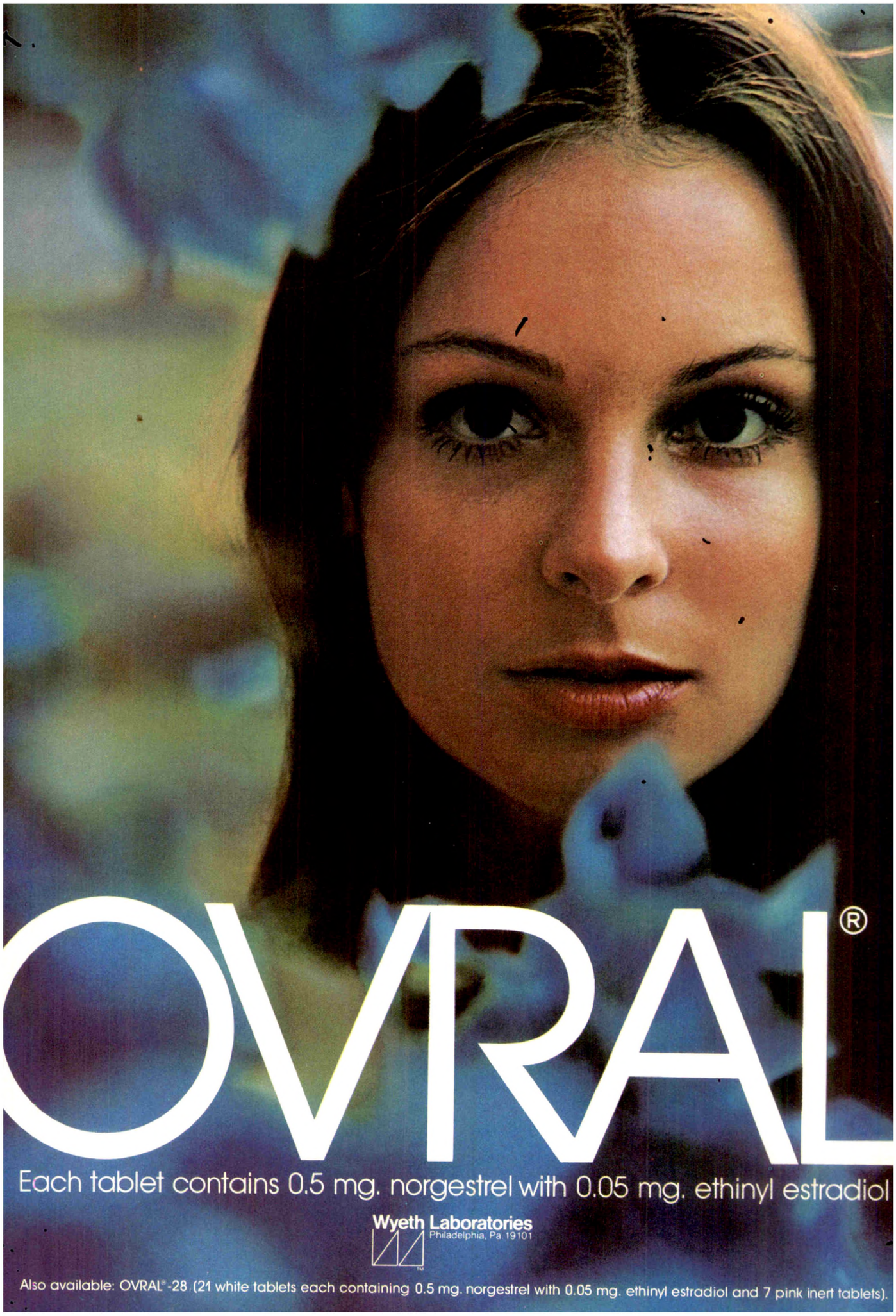
We cannot, on the basis of our data, recommend routine culturing of mothers during pregnancy as a standard clinical procedure. Additional data are clearly needed to determine whether successful eradication can be obtained by the methods suggested above. Benzathine penicillin was used successfully to eradicate streptococci from the vaginal flora of 13 women studied by Franciosi and associates.[4] There are no data on follow-up cultures from that population; however, this would seem to be a logical approach in a continued investigation. Ampicillin was chosen for the present study because of data from Bergquist and co-workers which suggested that a significant number of strains of streptococci were partially resistant to penicillin. All of the strains encountered in the present study were sensitive to penicillin. There are no published reports of penicillin-resistant group B streptococci in the United States.

Although group B streptococcal colonization of infants was not found to be influenced by maternal antibiotic treatment with ampicillin, temporary eradication of the flora in the birth canal was demonstrated. Streptococcal sepsis continues to grow as a significant cause of neonatal death and morbidity. Data from our institution and those of others have not shown that antibiotic treatment of the infant at the onset of symptoms is successful. Routine prophylactic treatment of colonized infants is unlikely to be satisfactory because of the frequent rapid onset of the disease in the first 48 hours of life prior to the availability of culture results. Therefore, continued efforts at maternal identification of group B streptococcal carriers and an additional trial of antibiotic therapy in controlled conditions are indicated.

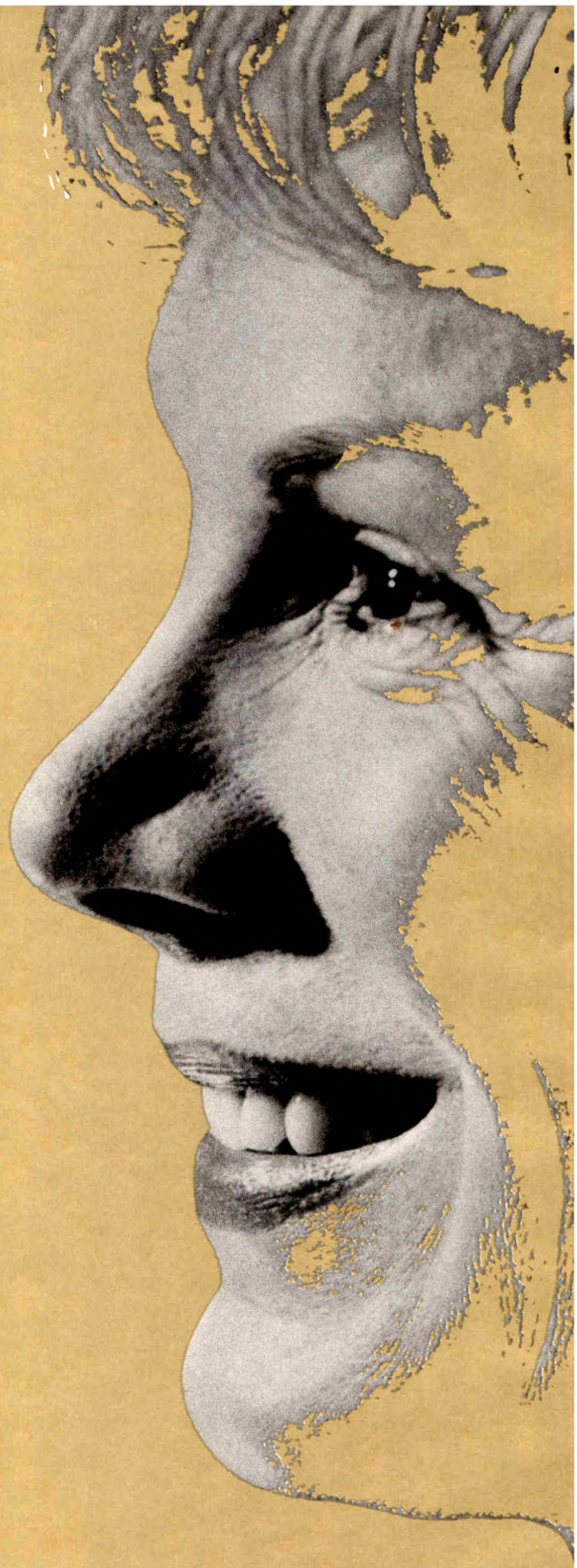
The authors would like to express their appreciation to the house staff and nursing personnel of the Kansas City General Hospital for their participation in the study.

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cyclic therapy recommended

Recently, published reports of retrospective human data have emphasized that patients using estrogens may have an associated increased risk of endometrial cancer. This information makes cyclic rather than continuous estrogen replacement therapy even more prudent. (See brief summary.)

With Estrace, treatment should be initiated with a dose of 1 or 2 mg. daily and then adjusted to the lowest dose possible still maintaining the relief of symptoms. The usual regimen for cyclic administration of Estrace is three weeks on drug, followed by one week off drug. Some patients may require a slightly shorter estrogen-free interval (4–6 days) to prevent recurrence of menopausal symptoms.

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well accepted by patients[1]

In the 369 patients previously described, the incidence of estrogen-induced side effects with Estrace was relatively low. Incidence of nausea was remarkably low—only 2% of patients before dosage adjustment. Some patients who had complained of this problem while on conjugated equine estrogens reported its complete disappearance on changing to Estrace. Whereas before dosage adjustment 24% experienced breast tenderness, 31% edema, 18% (of 151 with intact uteri) bleeding, and 4% weight gain, only about 2% of the 369 patients dropped out because of side effects.[1]

long-term therapy should be monitored

A few instances of significant gynecologic disease became apparent during clinical investigations.[1] Endometrial hyperplasia was discovered in seven patients, all of whom had previously received estrogen therapy for one to three years prior to entering the study. One patient was reported to have carcinoma *in situ* and another showed adenocarcinoma of the endometrium. In clinical practice, the physician should be alerted to the potential of thrombotic disorders and be prepared to discontinue medication immediately if they occur. Because of variations in normal endogenous hormone production, certain patients may exhibit signs of excessive estrogenic stimulation. Adverse reactions generally associated with estrogen administration may occur and these are listed in the brief summary. Prior to initiation of treatment with Estrace or any other estrogenic preparation, an adequate physical examination should be given.

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For Brief Summary, please see following page.

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Indications: Estrace® (17β-Estradiol) is indicated for relief of endogenous estrogen deficiency and associated menopausal symptoms.

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Warnings: The physician should be alert to the earliest manifestations of thrombotic disorders (thrombophlebitis, cerebrovascular disorders, pulmonary embolism and retinal thrombosis). If these occur or are suspected the drugs should be discontinued immediately. Discontinue medication pending examination if there is a sudden onset of proptosis, diplopia, or migraine. If examination reveals papilledema or retinal vascular lesions, medication should be withdrawn. Estrogens may be excreted in the mother's milk and an estrogenic effect upon the infant has been described. The long range effect on the nursing infant cannot be determined at this time. Hypercalcemia may occur in as many as 15% of breast cancer patients with metastases and this usually indicates progression of bone metastases. This occurrence depends neither on dose nor on immobilization. In the presence of untoward effects such as progression of the cancer or hypercalcemia, estrogen medication should be stopped. A statistically significant association has been reported between maternal ingestion during pregnancy of diethylstilbestrol and the occurrence of vaginal carcinoma developing years later in the offspring. Whether such an association is applicable to all estrogens is not known at this time. In any event, estrogens are not indicated for use during pregnancy.

Precautions: Because normal endogenous hormone production varies individually, certain patients may be unusually responsive to estrogenic therapy and may respond with undesirable manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding, mastodynia, edema, etc. Because of estrogen induced salt and water retention, these drugs should be used with caution in patients with epilepsy, migraine, asthma, cardiac or renal disease. Patients with a history of psychic depression should be carefully observed and the drug discontinued if the depression recurs to a serious degree. In the event that any unexplained or excessive vaginal bleeding would occur while on estrogen therapy, nonfunctional causes should be borne in mind. The drug should be discontinued and a thorough investigation made as to the cause, being certain to rule out the possibility of malignancy. Pre-existing uterine fibromyomata may increase in size while using this product, therefore, patients should be examined at regular intervals while receiving estrogenic therapy. Women with a strong family history of cancer, recurrent chronic cystic mastitis, or abnormal mammograms should be administered estrogens with caution. Because of a possible decrease in glucose tolerance, diabetic patients should be followed closely. Because estrogens influence the metabolism of calcium and phosphorus, they should be used with caution in patients with certain metabolic bone diseases that are associated with hypercalcemia or in patients with renal insufficiency. The pathologist should be advised of estrogen therapy when relevant specimens are submitted. Because of the effects of estrogens on epiphyseal closure, they should be used judiciously in young patients in whom bone growth is not complete. A pre-treatment physical examination should include special reference to breast and pelvic organs as well as a Papanicolaou smear since estrogens have been known to produce tumors, some of them malignant, in five species of animals. When large doses of estrogens are used, urinary stress incontinence may occur in nonpregnant females. Prolonged high doses of estrogens will inhibit anterior pituitary functions. This should be borne in mind when treating patients in whom fertility is desired. Continuous use of estrogens will result in prolonged stimulation of the endometrium and breast. In order to avoid this, oral estrogens should be administered cyclically in the menopausal or hypogonadal patient. The age of the patient constitutes no absolute limiting factor, although treatment with estrogens may mask the onset the climacteric. Certain endocrine and liver function tests may be affected by treatment with estrogens. If such tests are abnormal in a patient taking these drugs it is recommended that they be repeated after the drug has been withdrawn for two months. Any possible influence of prolonged estrogen therapy pituitary, ovarian, adrenal, hepatic, or uterine function awaits further study.

Adverse Reactions: A statistically significant association has been demonstrated between use of estrogen-progestin oral contraceptives and the follow serious reactions: Thrombophlebitis, pulmonary embolism, and cerebral thro bosis. Although available evidence is suggestive of an association, such a rel tionship has been neither confirmed nor refuted for the following serious reac tions: coronary thrombosis and neuro-ocular lesions (e.g. retinal thrombosis and optic neuritis). The following adverse reactions are known to occur in patients receiving estrogens: nausea, vomiting, anorexia, gastrointestinal symptoms (such as abdominal cramps or bloating), edema, breakthrough ble ing, spotting or withdrawal bleeding, breast tenderness and enlargement, cha in body weight (increase or decrease), headache, increased cervical mucus, allergic rash, loss of libido and gynecomastia in the male, reactivation of endometriosis, aggravation of migraine headaches, hepatic cutaneous porphyri becoming manifest, cholestatic jaundice, rise in blood pressure in susceptibl individuals, mental depression, cystitis-like syndrome, loss of scalp hair, erythema nodosum, hemorrhagic eruption, premenstrual-like syndrome, changes in libido, changes in appetite, nervousness, dizziness, fatigue, backache, erythema multiforme, itching, possible diminution in lactation when giv immediately post-partum, irritability, and malaise.

Important Notes: An increased risk of thromboembolic disease associated v the use of oral contraceptives containing estrogens and progestins has now been conclusively established. Retrospective studies have shown a statistica significant association between thrombophlebitis, pulmonary embolism, and cerebral thrombosis and embolism and the use of these drugs. There have be three principal studies in Great Britain[1-3] and one in the United States[4] leadin this conclusion. As a result of these studies, it has been estimated that users oral contraceptives containing estrogens are 4 to 7 times more likely than no users to develop thromboembolic disease without evident cause. The Americ study also indicated that the increased risk did not persist after discontinuati nor was it enhanced by long continued administration. Although the America study was not designed to evaluate a difference between products, it did sug that there might be an increased risk of thromboembolic disease in users of s quential products. Confirmation of this finding requires further study. In a mo recent analysis of data derived from several national adverse reaction reporti systems,[5] British investigators concluded that the risk of thromboembolism, cluding coronary thrombosis, is directly related to the dose of estrogen used oral contraceptive products. Their analysis did suggest, however, that the qu tity of estrogen may not be the sole factor involved. Nevertheless, in view of t study, as well as others that have demonstrated a positive relationship betwe estrogens and thromboembolism, it would seem prudent and in keeping with basic therapeutic principles, to utilize, whenever feasible, the smallest effecti dose of estrogen in treating patients. Risks associated with certain other kno adverse reactions, such as elevated blood pressure, liver dysfunction, and reduced tolerance to carbohydrate, have not as yet been quantitated. Long te administration of both natural and synthetic estrogens in subprimate animal species in multiples of the human dose increases the frequency for some ani carcinomas. These data cannot be transposed directly to man. The possible c cinogenicity due to the estrogens can neither be confirmed nor refuted at this time. Close clinical surveillance of all women taking estrogens must be continued. It has been reported in a recent study done in the United States[6] that maternal ingestion of diethylstilbestrol during pregnancy appears to increase risk of vaginal adenocarcinoma developing years later in the offspring expose

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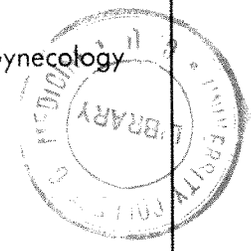
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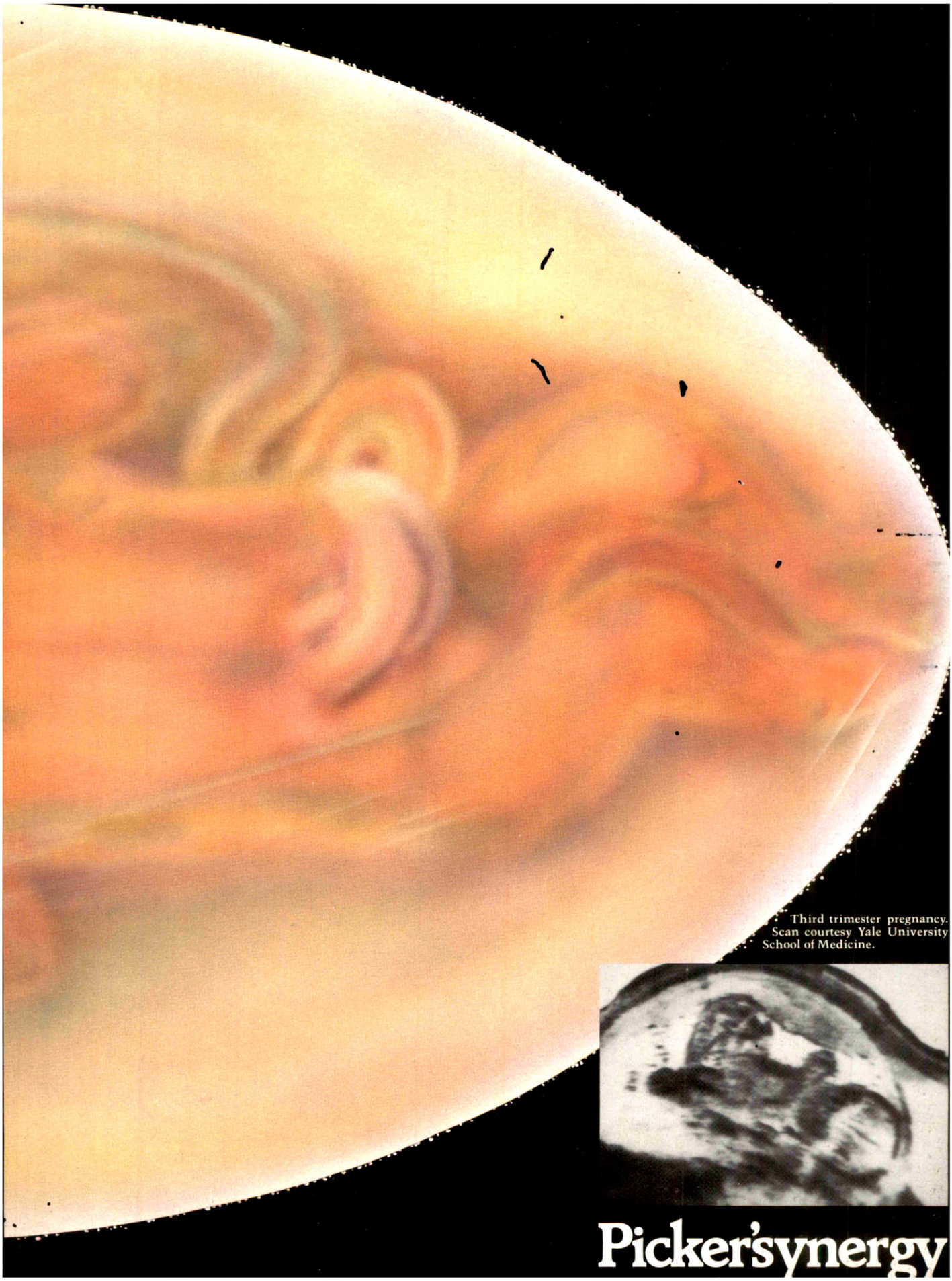
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FETUS, PLACENTA, AND NEWBORN

Cytogenetics of methadone-managed and heroin-addicted pregnant women and their newborn infants

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Chromosomal damage was assessed prenatally and at delivery from 99 addicted pregnant women (80 from a methadone maintenance program and 19 heroin addicts) and their 101 offspring at delivery. About 10 per cent of the 27,907 cells scored showed chromosomal aberrations. Chromosome damage was random, affected all chromosomes, and was mainly of the acentric fragment type. The per cent of hypodiploidy was significantly higher than the per cent of hyperdiploidy. In the mothers, no significant differences were found with respect to dosage and duration of methadone treatment and years of heroin abuse. No significant association was found between maternal variables and infant chromosome damage. Infants with low Apgar scores (1 to 6) had cells with significantly higher levels of chromosome damage than infants with higher scores (7 to 10).

CONCERN ABOUT the potential genetic hazards of short- and long-term exposure to drugs of abuse has motivated efforts to identify those compounds associated with cytogenetic damage as well as to examine the possible effects on the organism of such damage. Some investigators have attributed chromosome alterations to drug abuse,[1, 2] while others have not observed this relationship.[3–6] A multiplicity of factors, such as chronic fatigue, poor health, and inadequate nutrition, usual concomitants of drug intoxication which may themselves be related to chromosome damage, compound the difficulties encountered in such investigations and may have contributed to the contradictory results reported.[1–6]

The substitution of the synthetic narcotic drug methadone for heroin addiction, especially its prescription during pregnancy, raises anew the cytogenetic questions long associated with illicit drug abuse. Residual chromosome damage has been reported in former heroin addicts before and during methadone treatment,[7] but in vitro methadone cultures of newborn cord blood did not reveal an increase in chromosome damage.[8] The primary objective of this report is to consider the qualitative and quantitative cytogenetics of peripheral blood cultures from methadone-managed and heroin-addicted pregnant women and from the resulting offspring at birth.

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Table I. Summary chromosome profiles

Type of sample	*No.*	*Aberrant cells (%)*		*Aberrations/ aberrant cell*	*Aberrations/ 100 cells scored*	*Chromatid breaks (%)*	*Chromatid lesions* (%)*
Maternal prenatal	80	12.01†	10.05	1.44	14.11	6.25	2.33
		(0.63‡)	(0.60)	(0.11)	(1.02)	(0.42)	(0.30)
Methadone	76	12.10	10.17	1.45	14.31	6.33	2.31
		(0.66)	(0.62)	(0.12)	(1.06)	(0.43)	(0.31)
Heroin addicts	4	10.25	7.75	1.31	10.25	4.75	2.75
		(1.93)	(2.14)	(0.04)	(2.98)	(1.55)	(0.25)
Maternal delivery	99	12.56	10.10	1.39	14.34	6.49	3.00
		(0.54)	(0.51)	(0.04)	(0.90)	(0.42)	(0.31)
Methadone	81	12.95	10.43	1.42	15.05	6.74	3.02
		(0.60)	(0.58)	(0.04)	(1.03)	(0.48)	(0.37)
Heroin addicts	18	10.83	8.61	1.25	11.11	5.39	2.72
		(1.09)	(1.07)	(0.04)	(1.52)	(0.81)	(0.49)
Infant delivery	101	12.48	9.87	1.36	14.10	6.38	3.20
		(0.67)	(0.54)	(0.03)	(1.01)	(0.39)	(0.39)
Methadone	82	12.52	9.73	1.36	14.03	6.34	3.38
		(0.77)	(0.61)	(0.03)	(1.18)	(0.43)	(0.46)
Heroin addicts	19	12.32	10.47	1.36	14.37	6.58	2.42
		(1.34)	(1.21)	(0.05)	(1.88)	(0.92)	(0.57)

*Gaps.
†Includes lesions.
‡Standard error.

Material and methods

In response to an urgent need for information concerning the effects of narcotic addiction and methadone maintenance on pregnancy and the immediate postpartum period, the Departments of Obstetrics and Gynecology, Psychiatry, Pediatrics, and Medicine and the Illinois Drug Abuse Program instituted a multifaceted program of clinical and research investigations. The course of pregnancy in methadone-managed and heroin-addicted women was studied for any possible effects of narcotics.

Addicts were from the Chicago metropolitan area; 102 obtained methadone prenatally at different times during the pregnancy through the Illinois Drug Abuse Program (I.D.A.P.), and 21 women were heroin addicts who presented at the Chicago Lying-in Hospital only at delivery. Study patients were primarily Black (86.2 per cent), Caucasian (8.9%), Spanish (4.1 per cent), and other (0.8 per cent). The mean age was 25.1 years with a range of 16 to 44 years. Forty-two per cent of the women were between 21 and 25 years of age. There was an average of 1.9 living children per patient.

The addiction histories, obtained by the cytogenetics staff, revealed heroin to be the primary drug of abuse for more than three years in 74.5 per cent of the population. Multiple-drug abuse was common for all subjects and included alcohol, marihuana, cocaine, and other hypnotics, sedatives, stimulants, and depressants. Illicit drug use during pregnancy for those on methadone maintenance was monitored by I.D.A.P. The methadone dosage range for those in prenatal treatment was 0 to 80 mg. daily with an average daily dose of 32.7 mg. The times of entry into the I.D.A.P. were: prior to conception, 41.5 per cent; first trimester, 9.8 per cent; second trimester, 17.9 per cent; third trimester, 13.8 per cent; never entered (heroin addicts), 17.1 per cent.

Cytogenetic samples were obtained from 80 methadone-managed women and 19 heroin-addicted women. A blood sample was drawn at the first clinic visit for those women who had prenatal visits. At delivery, samples of maternal and infant cord blood were obtained from all patients. All deliveries were attended by the cytogenetic staff, and all cultures were started immediately after the blood was obtained. In no instance was blood refrigerated for later culturing. Nearly all cultures had 100 cells scored for a total of 27,907 cells. Preparation of 72 hour blood cultures with the use of a whole blood micromethod, harvesting, scoring of the chromosome complements, and classification of chromosome aberrations were carried out as previously described.[9] All slides were coded so that the observer was unaware of any relationship to patients. To strengthen the impartiality, no observer continually scored slides from the spaced peripheral blood cultures of the same subject.

The ploidy and number and type of aberrations in each cell scored were entered onto forms which permitted direct keypunching. Chromosome profiles for each sample, as well as statistical analyses, were

Chromosome aberrations (%)	*Aneuploidy (%)*	
	Hypodiploidy	*Hyperdiploidy*
4.43	4.00	1.23
(0.34)	(0.27)	(0.14)
4.50	4.09	1.21
(0.36)	(0.28)	(0.14)
3.00	2.25	1.50
(0.71)	(0.25)	(0.87)
4.32	4.78	1.17
(0.28)	(0.30)	(0.12)
4.44	4.73	1.12
(0.32)	(0.34)	(0.14)
3.78	5.00	1.39
(0.63)	(0.67)	(0.24)
4.12	5.84	1.37
(0.29)	(0.42)	(0.18)
4.10	6.17	1.38
(0.33)	(0.49)	(0.21)
4.21	4.42	1.32
(0.49)	(0.64)	(0.25)

compiled with the Xerox Sigma V computer. Significance was based on the Student's t test and Pearson correlation-coefficient statistics.

Results

Summary chromosome profiles for all study cases subdivided by type of sample, as well as maternal drug of abuse, are presented in Table I. About 10 per cent of all cells scored had some type of aberration. This figure did not differ much among the maternal prenatal (10.05), maternal delivery (10.10) and newborn (9.87) samples. If cells with chromosome lesions (gaps) are also considered as damaged, the per cent of aberrant cells increases to 12.4 per cent, with all the types of samples undergoing a similar increase. The women not in the methadone program had a slightly lower per cent of aberrant cells than methadone-managed women, but this difference was not statistically significant.

Although examination of the per cent of aberrant cells does provide information as to the incidence of damaged cells, it does not quantify the extent of damage in an aberrant cell. For this purpose the ratio of the total number of aberrations to the total number of aberrant cells and to the total number of cells scored for each subject was computed. Although the number of aberrations per total cells scored does not alone permit isolation of extent of damage in an individual cell, it is a commonly used index which allows comparison of the present results to those reported in the literature. On the average, there were about 14 aberrations for every 10 aberrant cells scored. This remained fairly constant for the different samples. However, the heroin-addicted women at delivery had a significantly lower rate of aberrant cells (1.25) than the methadone-managed group (1.42). There was also a statistically significant difference between these two groups at delivery when aberrations per total cells scored were considered. The heroin addicts had a rate of 11 per cent while those on methadone had a rate of 15 per cent. It is of interest to note that the chromosome damage of infants in the two groups did not differ as measured by any of the indices.

When aneuploidy was determined, the per cent of hypodiploid cells was significantly higher than the per cent of hyderdiploid cells for all three sample types. The per cent of hypodiploidy was significantly higher in the newborn infants when compared to the two types of maternal samples.

The extent to which infant chromosome damage can be predicted from maternal prenatal and/or delivery samples is also of particular interest. Fig. 1 is a scatterplot of the maternal delivery damage versus infant cord blood damage. Each of the points on the graph represents one or more mother-infant pairs. If there was a perfect association between maternal and infant damage, the points would cluster closely about a straight line or some other type of curve. As can be seen from Fig. 1, the points show a large amount of variability which cannot be explained by fitting any type of curve. However, even if the association between maternal and infant damage is not very strong, the question as to whether any degree of association exists can be considered. With the correlation coefficient, a measure of linear association, no relationship was found between the per cent of aberrant cells of the mother and newborn infant at delivery. When the per cent of aberrant cells including chromosome lesions was considered, there was a small ($r = 0.26$) but statistically significant ($p < 0.01$) association between maternal delivery and newborn damage. There was also a significant association between delivery maternal and newborn hypodiploidy ($r = 0.25$; $p < 0.01$) and diploidy ($r = 0.25$; $p < 0.01$). When the relationship between maternal prenatal and newborn samples was examined, no significant associations were found. The association between the two types of maternal samples was also weak, though statistically significant, for the per cent of aberrant cells without lesions ($r = 0.26$; $p < 0.05$), the per cent of aberrant cells with lesions ($r = 0.19$; $p < 0.05$), and the per cent of hyperdiploid cells ($r = 0.19$; $p < 0.05$).

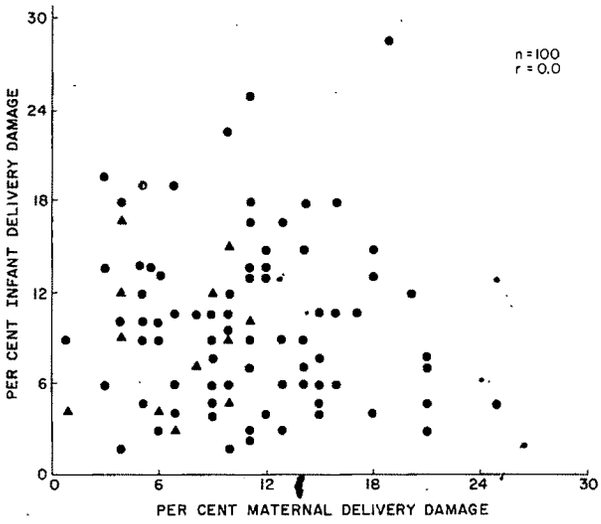


Fig. 1. Relationship at delivery between per cent of maternal and infant cells with chromosome damage. Symbols representing the number of mother-infant pairs are: • = 1; ▲ = 2.

Also considered was whether poor infant outcome—whether it be depressed Apgar scores, intrauterine growth retardation, or presence of withdrawal symptoms severe enough to necessitate chemotherapeutic intervention—was also associated with an increase in chromosome damage. Only Apgar scores appeared to have some type of association with chromosome damage (Table II). Infants with low Apgar scores had more chromosome damage than infants with high Apgar scores. There was a significant difference in the number of aberrations per aberrant cell for infants with one- and five-minute Apgar scores of 6 or less when compared to infants with scores of 7 to 10. Infants were also subdivided by a variety of maternal characteristics which might affect infant chromosome damage: duration of time heroin was used as a primary drug, type of primary drug at conception and delivery, and duration and dosage of methadone treatment. Infants born to mothers addicted to heroin appeared not to differ significantly from infants born to methadone-managed mothers. No consistent difference with respect to duration of reported maternal heroin usage or methadone dosages could be found. Both the maternal prenatal and delivery samples were subdivided by the same characteristics as the infant samples. Again, no clear-cut differences emerged with respect to dosage, duration of methadone treatment, and years of heroin use, although, as shown in Table I, the women who did not enter the methadone program had fewer aberrant cells. When particular types of chromatid and chromosome aberrations were scored, the prevalent type in all cells was the chromatid break and lesion. Chromosome damage (involving both sister chromatids) was predominantly that of the isochromatid break (ace). There were no standard abnormal karyotypes, clones of abnormal cells, or marker chromosomes. Chromosome damage was random and involved all chromosones.

Table II. Chromosome profile of infants subdivided by Apgar scores

Apgar score	*No.*	*Aberrant cells (%)*		*Aberrations/ aberrant cell*
One minute				
1-3	4	20.0*	17.5	1.83
		(5.71†)	(4.44)	(0.15)
4-6	8	14.5	11.2	1.43
		(2.70)	(2.53)	(0.13)
Total	12	16.3	13.3	1.57
		(2.58)	(2.30)	(0.11)
7-8	32	12.6	9.6	1.39
		(1.30)	(0.95)	(0.05)
9-10	57	11.6	9.3	1.30
		(0.75)	(0.61)	(0.03)
Total	89	12.0	9.4	1.33
		(0.67)	(0.52)	(0.03)
Five minute				
1-3	1	36.0	29.0	2.28
		(–)	(–)	(–)
4-6	5	11.0	10.4	1.60
		(2.97)	(2.87)	(0.06)
Total	6	15.2	13.5	1.72
		(4.82)	(3.89)	(0.12)
7-8	6	16.7	13.2	1.28
		(2.39)	(2.14)	(0.07)
9-10	89	12.0	9.4	1.34
		(0.67)	(0.53)	(0.03)
Total	95	12.3	9.6	1.34
		(0.66)	(0.52)	(0.03)

*Includes lesions.
†Standard error.

Comment

In view of the difficulties encountered with the identification of in vivo chromosome-altering agents, progression to yet another generation, the newborn infant, compounds the already complex problem. When the infant is of drug-addicted parentage, the number of possible variables which might influence chromosome damage increases greatly, especially since there have been no similar studies in nonaddict mother/infant pairs. The degree of maternal prenatal and delivery chromosome damage explained very little of the variation observed in the newborn infant, suggesting several possible mechanisms. For example, the factors which cause chromosome damage may affect the mother and infant differently. Although at conception the levels of maternal and fetal damage may have been comparable, continued exposure of both to varied stresses may have resulted in differing chromosome profiles at delivery. The weak association between maternal samples prenatally and at delivery

indicates that maternal chromosome damage did not remain stable throughout the prenatal period but fluctuated over relatively short time periods. Whether a similar situation would exist in drug-free pregnant women remains unknown. The failure to establish an association between the level of the various drug-related variables and the degree of chromosome damage may suggest there is none.

Equally likely is the possibility that numerous complex interactions of both measured and unmeasured variables obscured the examined relationships. Perhaps under more carefully monitored life-style conditions different results would have been obtained. It must also be emphasized that since nonaddict infant cord blood and maternal measurements are unavailable it cannot be established whether our reported values are significantly different from those that would be observed in matched control subjects.

The increased incidence of chromosomal damage in infants with low Apgar scores suggests that elevated chromosome damage may be concomitant with differing types of neonatal difficulties associated with depressed Apgar scores. The exact role of the observed chromosomal damage in such situations is unknown. It is conceivable that chromosomal damage may have led to the neonatal difficulties or that the difficulties could have contributed to the increased damage.

Although no discernible differences in infant chromosome profiles were seen between infants requiring treatment for withdrawal and those who did not, the very small number of infants judged to have no withdrawal and delayed onset of withdrawal in many, as well as the possibility of previous in utero withdrawal, makes any definitive conclusions tenuous.

The reported frequency of aberrant cells in drug users and control subjects has ranged from 1 to 20 per cent.[8, 14, 15] In this study the incidence of the particular types of chromosome damage was lower in all but one category (isochromosomes) when compared to the evaluations of 2,237 cells from former illicit drug users.[6] Although the over-all per cent distribution of isochromosomes was slightly higher, there were only two ring chromosomes observed in the 27,907 cells scored.

The biological significance of chromatid gaps is not known, but continued notation of these appears required in view of evidence of association of specific diseases with specific and consistent achromatic gaps. Three patients with congenital erythroid hypoplasia (Blackfan-Diamond syndrome) and an abnormality of chromosome no. 1 have been reported.[10–13] One patient had an achromatic gap in the same region of one arm of Chromosome 1.[10, 11] The patients of Gray and associates[12] and Heyn and colleagues[13] also had an abnormality in the same chromosome. These three studies relate the chromatid gap to a specific disease. In this study we originally included chromatid lesions in Fig. 1, and the relationship of maternal delivery/infant delivery chromosome damage was statistically significant. When the chromatid gaps were removed, the results did not show statistical significance.

One of the principal concerns in drug abuse is the possible production of irreversible chromosome damage which could be deleterious. Although chromosomal changes in general are not pathognomonic of narcotics abuse, the increased incidence of chromosomal abnormalities in the lymphocytes from narcotics abusers when compared to non–drug users strengthens the possibility of a relationship. The life-styles of the 99 subjects did not demonstrate a single instance of aseptic administration of the drugs, and there is no way we can prove the purity of the preparations. Compounding the drug addiction was the presence of poor nutrition, lack of rest, stress, increased incidence of tuberculosis, hepatitis, and syphilis, and a lack of early and consistent prenatal care. For these reasons as well as simultaneous poly–drug use, it is impossible to isolate either methadone or heroin as the causative agent. Conceptually, it is possible that any of these factors could have damaged the detoxifying potential of the liver to such an extent that the small lymphocytes became "fragile," "infected," or "primed" so that in vitro stimulation into mitosis either elicited existing residual chromosomal damage or engendered chromosome damage in these weakened cells within the in vitro milieu. In summary, the biological implications of chromosome damage cannot be completely understood until it can be ascertained how the human subject reacts to chromosome damage when there is no evidence of clinical or pathologic syndromes.

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Placental morphometric and morphologic alterations in maternal undernutrition

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Placentas, mothers, and neonates belonging to different socioeconomic groups were examined for morphometric and gross morphologic changes to assess the effect of maternal undernutrition. The mean maternal caloric intakes for socioeconomic Groups I, II, and III + IV are 2,919, 2,420, and 1,589 calories per day, respectively, and differences were significant ($P < 0.001$). The mean protein intakes were respectively 76.1, 67.3, and 52.0 Gm. per day for socioeconomic Groups I, II, and III + IV, respectively ($p < 0.001$). The birth weight, placental weight, volume, surface area, and number of cotyledon showed significantly decreasing trends ($P < 0.001$) with the fall in socioeconomic group and maternal dietary intakes. Placentas of socioeconomic Group I had significantly higher incidence of well-defined cotyledons and central cord attachment. In lower socioeconomic groups and small-for-dates placentas there was significantly higher incidence of hemorrhages. There were no changes in incidences of placental infarcts, calcifications, and degeneration in various socioeconomic groups.

CLINICAL observations indicate that babies born during calamities like famine or persistent nutritional deprivation generally weigh less in relation to their gestational age.[1-3] Smith,[4] in a post-war study in Holland, noted that dietary deficiencies due to rationing, especially if this coincided with the last trimester of pregnancy, affected the fetal growth. Similar studies by Dean[5] in Germany demonstrated an average reduction of birth weight by 118 Gm. in relation to gestational age which was attributed to undernutrition of the pregnant mothers.

In a recent study, Laga and associates[6] demonstrated that the placentas of low socioeconomic group mothers had anatomic, morphologic, and histologic alterations. He compared Bostonian and Guatemalan pregnant mothers as upper and lower socioeconomic material.

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The aim of the present study is to observe placental morphometric and morphologic alterations during maternal nutritional deprivation.

Material and methods

Plan of study. The work spread over a period of 11 months—from February 1, 1973, to December 31, 1973. About 150 sets of mothers, babies, and placentas were selected at random for the study from the Neonatology Unit, Department of Pediatrics, Institute of Medical Sciences, Varanasi. Out of these, 47 sets were taken as controls belonging to socioeconomic Group I; the remaining 103 sets were from socioeconomic Groups II and III + IV (occupational classification of Government of India, 1962).[7] This material from the district of Varanasi had no racial, cultural, or environmental differences. No subject with obstetric abnormalities, i.e., abruptio placentae, toxemia, twins, diabetes, hypertension, congestive cardiac failure, etc., was included in this study.

Methodology. Socioeconomic, dietetic (according to the Indian Council of Medical Research, New Delhi, questionnaire method),[8] and obstetric histories were obtained. Clinical examination was also performed to

Table I. Distribution of newborn infants, placentas, morphometry, and maternal calorie and protein intakes in differe

Socioeconomic groups	Appropriate for gestational age		Small for dates	Material dietary intake per day		Placenta	
	Term	Preterm		Calorie	Protein	Weight (Gm.)	Volume (ml.)
I	41	6	—	2,919 ± 21.4	76.1 ± 3.1	499 ± 65.4	423 ± 34.8
II	38	4	11	2,421 ± 103.0	67.3 ± 2.0	320 ± 34.2	328 ± 40.5
III + IV	3	9	38	1,590 ± 101.4	52.1 ± 2.1	287 ± 27.3	263 ± 17.8
P values:							
I vs. II				<0.001	<0.001	<0.001	<0.001
II vs. III + IV				<0.001	<0.001	<0.05	<0.001

Table II. Placental morphometric data in relation to newborn status (mean with standard deviations)

Newborn status	No.	Placenta			No. of cotyledons	Newborn (Gm.)	Newborn/ placental weight ratio
		Weight	Volume	Surface area cm^2			
1. Appropriate for gestational age (term)	82	416 ± 75.4	402 ± 37.3	226 ± 26.2	19 ± 2.1	2,915 ± 75.5	7.0
2. Small for dates	49	296 ± 32.8	272 ± 31.6	168 ± 25.5	11 ± 2.0	2,371 ± 112.2	8.0
3. Preterm	19	268 ± 44.5	237 ± 42.5	153 ± 27.8	11 ± 3.4	1,787 ± 44.5	6.7
1 vs. 2		<0.001	<0.001	<0.001	<0.001	<0.001	
2 vs. 3		<0.02	<0.001	<0.05	0.90	0.001	

Table III. Placental gross morphologic changes in different socioeconomic groups (values given as percentages)

Characteristic	Socioeconomic groups			χ^2	P
	I	II	III + IV		
No.	47	53	50		
1. Ill-defined cotyledons	0.0	5.7	26.0	4.31*	<0.001
2. Eccentric attachment of the cord	29.9	74.5	94.4	59.87	<0.001
3. Presence of hemorrhages	2.1	44.7	48.2	29.57	<0.001
4. Presence of infarcts	25.0	36.2	33.5	1.68	>0.70
5. Presence of calcifications	35.4	16.7	13.0	6.49	>0.10
6. Presence of degeneration (in form of thinning of cotyledons)	31.3	53.2	48.2	5.16	>0.20

*Calculated by Student's t test.

exclude systemic diseases which could have affected the growth.

Placenta. Immediately after delivery the cord was cut flush with the placental surface and the membranes were trimmed off. Blood clots adhering to the maternal surface of the placenta were picked off and the subchorionic vessels were emptied of blood by gentle pressure. The placenta was blotted several times with filter paper and then weighed. The cord attachment, number and nature of cotyledons, and extent of infarcts, hemorrhagic areas, and evidence of degeneration in the form of thinning of cotyledons and presence of calcifications were recorded by one of us (L.S.M.).

Neonate. The gestational age of the newborn infants was assessed according to the criteria used by Usher and associates[9] and Robinson.[10]

Results

Placental parameters in relation to maternal socioeconomic status. The data in Table I showed that the number of small-for-dates babies is increasing in lower socioeconomic groups. The mean caloric and protein intakes were significantly different in various socioeconomic groups.

There was significant reduction in placental weight in socioeconomic Group II as compared to I. The fall

socioeconomic groups (means with standard deviations)

Surface area (sq. cm.)	*No. of cotyledons*	*Newborn weight (Gm.)*	*Newborn/ placental weight ratio*
216 ± 20.3	18 ± 2.1	2,820 ± 78.4	5.65
188 ± 18.5	15 ± 2.9	2,502 ± 138.4	7.83
169 ± 23.0	10 ± 1.6	2,261 ± 105.8	7.88
<0.001	<0.001	<0.001	
<0.001	<0.001	<0.001	

in group III + IV as compared to II was also possibly significant. The reduction in placental volume, surface area, and number of cotyledons in socioeconomic Group II as compared to I was significant and they further fell significantly in socioeconomic Group III + IV. The increase in the newborn placental ratios in the lower socioeconomic groups indicated greater fall in newborn weight as compared to placental weights.

Placental parameters and newborn weights in relation to maternal calorie and protein intakes. The relationships of maternal calorie and protein intakes per day with the placental parameters were studied by fitting the regression equations (Y = a + b). Significant linear relationships were observed for placental weight, volume, surface area, and number of cotyledons (Figs. 1 and 2).

The newborn weight significantly increased with higher maternal calorie intakes; the correlation coefficient (r) and the regression coefficient (b) were 0.58 and 0.49, respectively ($p < 0.001$). Similarly significant correlation was observed with maternal protein intake (r = 0.59; b = 25.17;–<0.001). The placental parameters and newborn weight did not differ significantly between protein intakes of 68 to 75 and 75 to 82 Gm. per day.

The data for appropriate-for-gestational-age babies showed that the placental weight, volume, surface area, and number of cotyledons were also significantly correlated with the weight of the neonate (Fig. 3). However, the small-for-dates and preterm placental weight, volume, surface area, and number of cotyledons did not have significant correlation with newborn weight. In these groups maternal mean calorie and protein intakes per day, placental weight, volume, surface area, and number of cotyledons and newborn weight were significantly lower than for appropriate-for-gestational-age group. The mean maternal calorie and protein intakes in the preterm group were significantly higher than in the small-for-dates group. In the preterm group newborn weight and placental parameters were lower than in the small-for-dates group. However, the number of cotyledons in the two groups remained unchanged (Table II).

The socioeconomic Group I placentas had a significantly higher incidence of well-defined cotyledons and central attachment of the cord. This group also showed a significantly lower incidence of hemorrhages. The small-for-dates and preterm placentas showed higher incidences for ill-defined cotyledons and hemorrhages than the appropriate-for-gestational-age group. These hemorrhages were in the form of small clots attached to the maternal surface of the placentas. The increase in incidences of placental infarcts, calcifications, and degenerations was related to increase in gestational age (Table III).

The means for maternal hemoglobin in Groups I, II, and III + IV were 11.30 ± 1.66, 9.64 ± 3.69, and 7.70 ± 1.77, respectively. The mean hemoglobin level in Groups III + IV was significantly lower than the means in Groups I and II. The placental weight, surface area, volume, and number of cotyledons as well as newborn weight showed a significant trend with the fall in maternal hemoglobin.

Comment

The data of the present study demonstrated that the mothers of the lower socioeconomic groups had inferior dietary intakes, reduced placental weight, and higher incidences of small-for-dates and preterm births. There was a proportionately greater fall in newborn weight than in placental weight. These observations are in conformity with those of other recent studies.[1, 2, 3, 6] The placental volume, surface area, and number of cotyledons were reduced in lower socioeconomic groups and showed significant relationship with calorie and protein intakes. The maximum weight gain was achieved on receiving 68 to 75 Gm. per day intake of protein and, with further protein in the diet, no extra gain in newborn weight was observed. The observations of the present study for placental and neonatal weights in the lower socioeconomic groups are supported by the findings of Laga and associates.[6] The mean placental volume for appropriate-for-gestational-age babies or of the mothers receiving over 68.0 Gm. of protein per day in the present study is in accord with the normal values reported by Ahern and Dunnill[11] and Garrow and Susan.[12]

To the present time, a reduced number of placental cotyledons has been demonstrated only in hypoxic placentas by Krugner and Stella.[13] The data of the

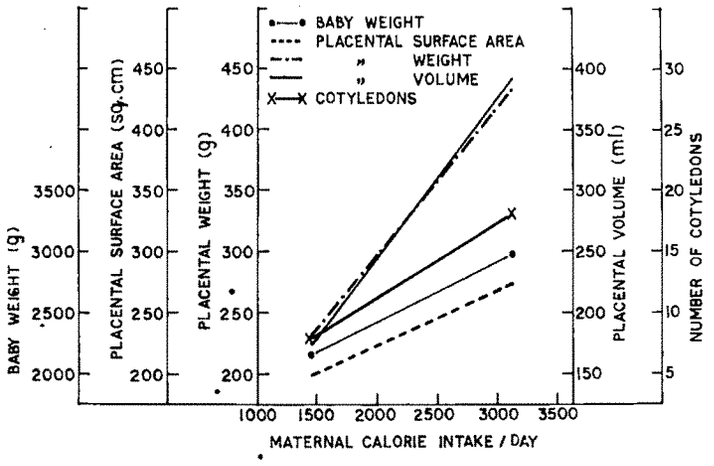


Fig. 1. Regression lines of maternal calorie intake and various dependent variables.

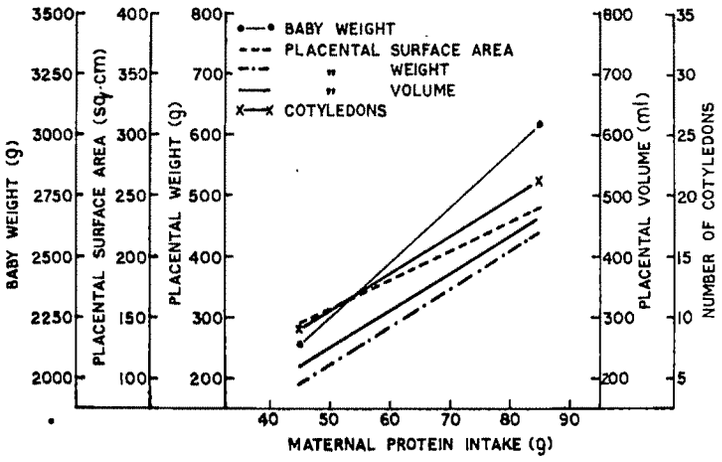


Fig. 2. Regression lines of maternal protein intake and various dependent variables.

present study demonstrated that in maternal undernutrition there is a significant reduction in the number of placental cotyledons. The data for the appropriate-for-gestational-age group are identical with those of upper socioeconomic group mothers who are receiving mean calorie and protein intakes of 2,723 ± 71.5 Gm. per day, respectively. The appropriate-for-gestational-age group data are significantly higher for all the parameters studied. The small-for-dates group data are higher than the preterm data for all parameters excepting the number of cotyledons, which remained unaltered. Desai and associates[14] reported reduced mean placental and neonatal weights for small-for-dates and preterm groups as compared to appropriate-for-gestational-age group. The mean number of cotyledons in preterm babies was around 11.3. This would mean that the number of cotyledons increased with gestational age and where for any reason fetal nutrition suffers, as in the case of small-for-dates babies. The increase in number of cotyledons does not follow the desired pattern; various maternal factors including undernutrition (demonstrated in the data of this study) and hypoxia (demonstrated by Krugner and Stella[13]) could be responsible.

Ahern and Dunnill[11] demonstrated reduced placental volume in abnormally small babies (350 ± 65) whereas the mean volume for small-for-dates placentas in the present study is 272 ± 32 ml., which is in accord with the findings of Pierre and associates.[15]

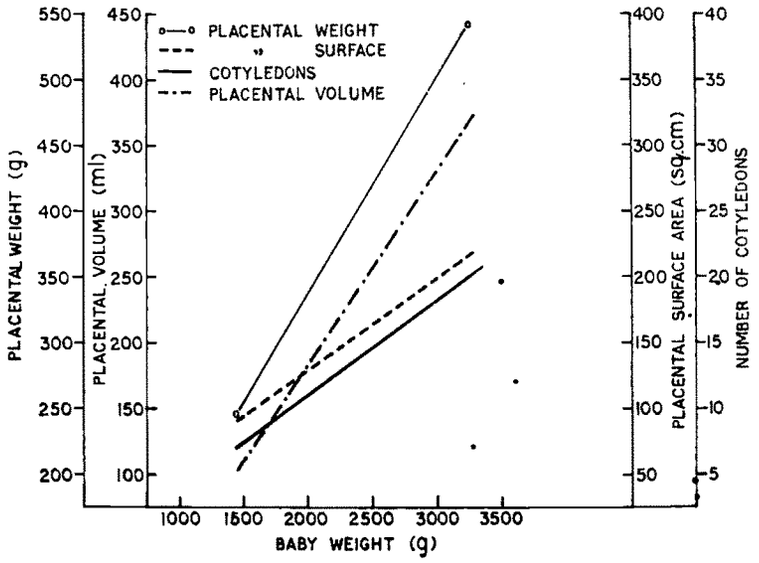


Fig. 3. Regression lines of baby weight in grams and various dependent variables.

Besides the fall in the placental cotyledon number, there is also an increase in the number of ill-defined cotyledons in the lower socioeconomic small-for-dates and preterm groups. The increase in ill-defined cotyledons has also been reported by Krugner and Stella[13] during hypoxia. This lesser degree of separation with greater degree of functioning parenchyma may be a compensatory phenomenon for transmitting gases and nutrients.

The incidence of eccentric attachment of cord increases in lower socioeconomic and small-for-dates groups, while the preterm appropriate-for-gestational-age placentas have mainly central attachments, which is in conformity with the findings reported by Laga and associates.[6]

The incidence of placental hemorrhages markedly increases in the lower socioeconomic and small-for-dates groups, which is in conformity with the findings of Desai and associates.[14] The increased incidence of placental hemorrhages has been associated with generalized protein and vitamin deficiencies.[16, 17] The number of infarcts, calcifications, and evidence of degeneration are not significantly different in the various socioeconomic groups. In contrast, Laga and associates[6] demonstrated increased incidence of old and recent infarcts, subchorionic fibrin, and cysts in lower socioeconomic groups. Desai and associates[14] found calcification and infarcts mainly in appropriate-for-gestational-age group and in a small percentage of small-for-dates placentas but none in the preterm. Fox[18] and Tindell and Scott[19] showed that the calcifications increased with gestation but Fujikura[20] demonstrated that fetal and placental weight variations did not significantly affect calcifications, which is in agreement with the findings of the present study. The degeneration seems to be a change occurring with increase in gestational age.

Whether placental morphometric and morphologic alterations in maternal undernutrition are partly or mainly due to hypoxia is difficult to comment upon in the present state of knowledge. The identical changes observed during hypoxia and relationship with maternal anemia do suggest such a possibility.[13, 19] Further studies on severe maternal anemia (hemoglobin < 5.0 Gm. per 100 ml.) are in progress and likely to throw light on this subject.

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Advanced course in colposcopy with video tape tutorial, July 22-24, 1976

An advanced course in colposcopy will be held during Montreal's Summer Olympics on July 22-24, 1976, at the Jewish General Hospital, Montreal, Quebec, Canada. Invited speakers include Drs. Duane Townsend, Arthur Herbst, Adolph Stafl, and Ralph Richart.

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Interactions of the trophoblast and maternal tissues in three closely related primate species

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The confluence of maternal and fetal tissues to form the placenta causes a wide variety of alterations in cells of both origins. Three closely related primate species were studied for similarities and differences in the interrelationships formed during various stages of placental development in the areas of implantation, decidualization, and trophoblastic involvement in the spiral arteries. Although certain similarities exist among the three species a surprising number of distinct differences were found to shed doubt on the common practice of assuming species-to-species similarity, particularly among primates.

THERE HAS BEEN increasing appreciation, in recent years, of the importance of the interaction of fetal and material tissues in pregnancy. Numerous far-reaching problems are involved in this mutual relationship, among the most important of which are: the achievement of implantation and the maintenance of pregnancy,[5] restraint of trophoblastic invasion,[15] control of uteroplacental circulatory anatomy and physiology,[10] and the paradoxical homograft reaction.[12]

The nature of the subplacental tissues has been studied in the human being,[7] the monkey (*Macaca mulatta*),[11] and the baboon (*Papio* sp.).[8, 9] In the course of these studies and in extensive review of the literature, certain dissimilarities among these three species have been noted which have fundamental significance. Although these observations do not of themselves provide solutions to the unresolved problems, they do when considered together supply certain suggestive data and clues which cannot be ignored and may assist in understanding placentation in general.

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Observations

The subplacental tissues of the human being, monkey (*Macaca mulatta*), and baboon (*Papio* sp.) were studied by conventional histologic staining techniques with the light microscope and by electron microscopy. For the purposes of this discussion, a comparison will be made between the three species with reference to the histologic behavior: (1) of the endometrial connective tissue ("decidualization") and (2) of the trophoblast, especially in its relation to the blood vascular system supplying the intervillous space.

Decidualization. Striking differences exist among the three species in the response of the endometrial tissue to implantation. In the human subject interstitial implantation occurs (Fig. 1, *a*), the embryo becoming completely embedded in the stroma with no notable proliferation of the uterine epithelium other than that necessary to recover the site of blastocyst penetration. However, the reaction of the endometrial connective tissue cells is extensive. The spindle-shaped cells begin to swell immediately after attachment of the blastocyst and become large polyhedral cells which almost obliterate the intercellular space (Fig. 2, *a*), especially around blood vessels.

In the rhesus monkey, on the other hand, the blastocyst remains superficially attached (Fig. 1, *b*). As it expands, the side opposite the initial attachment reaches the opposite wall of the uterus and attaches to

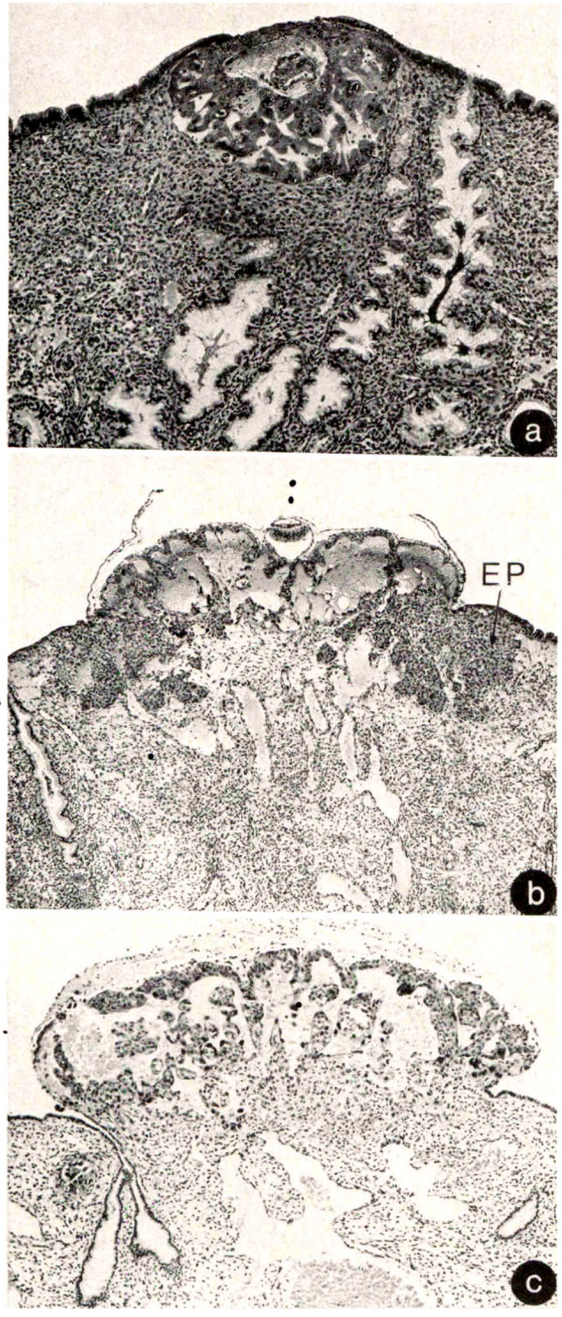


Fig. 1. Photomicrographs showing type of implantation in *(a)* the human subject (Carnegie No. 8004, section 11-4-7, ×100), interstitial, day 9; *(b)* the rhesus monkey (Carnegie No. C-652, section 4-3-8, ×100), superficial, day 13—note the epithelial plaque (EP); and *(c)* the baboon (No. A65-151, section 12-2-4, ×110), superficial, day 15.

it, forming a second placenta. The uterine epithelium in the area of attachment undergoes rapid and extensive proliferation, forming the well-known epithelial plaque, which persists for some 3 weeks. The

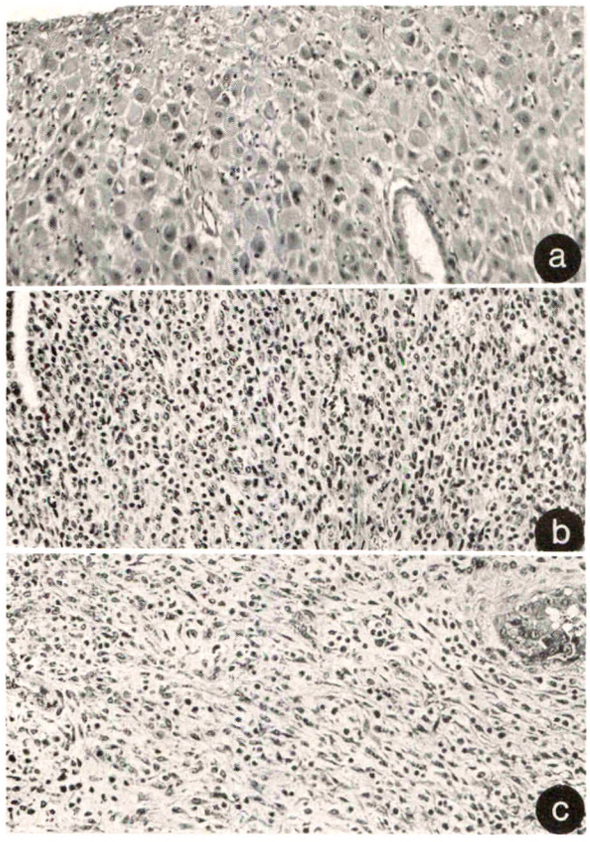


Fig. 2. Photomicrographs of subplacental endometrium in *(a)* the human subject, showing pronounced decidua (U. of Va. No. U6-3, ×120); *(b)* the rhesus monkey, no decidual reaction (Carnegie No. C-658, ×100); and *(c)* the baboon, no decidual reaction (No. A65-123, ×100).

connective tissue stroma does not undergo extensive cellular hypertrophy, although cells in a narrow band adjacent to the trophoblastic shell become somewhat swollen. This increase does not reach the proportions found in the human subject and no cell comparable to the decidual cell of man appears at any stage in this animal (Fig. 2, *b*).

The baboon blastocyst also remains attached superficially (Fig. 1, *c*) but neither an epithelial plaque nor a secondary placenta is formed. The cells of the endometrial stroma, like those of the rhesus monkey, undergo no extensive cellular hypertrophy (decidualization) in early stages (Fig. 2, *c*). Some cellular swelling, resembling that found in the rhesus monkey, is observed near the placenta, but again this does not reach the type of reaction occurring in the human.

Trophoblast. Just as the reaction of the maternal stroma to the presence of the blastocyst varies among the three species, so does the activity of the trophoblast. Common to the species is the presence of distinctive

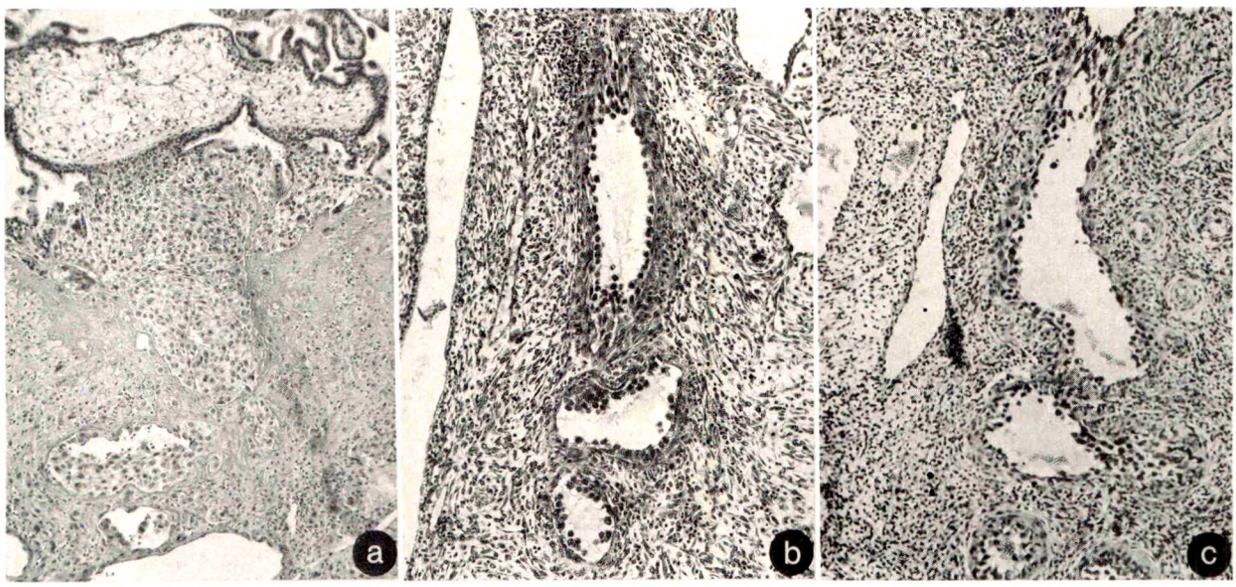


Fig. 3. Photomicrographs showing trophoblast in uteroplacental arteries, the "candle-wax dripping" phase, in (*a*) the human subject (U. of Va. No. U32-2, ×85); (*b*) the rhesus monkey (Carnegie No. 546, Section 16, ×100); and (*c*) the baboon (No. A65-177, section 38-2-1, ×40). Placental attachment is above in each instance.

Table I. Comparison of the main placental features of the rhesus monkey, human being, and baboon*

Man	*Monkey*	*Baboon*
Implantation:		
Interstitial	Superficial	Superficial
Pronounced decidual reaction	No decidual reaction	No decidual reaction
No epithelial reaction	Transitory epithelial plaque	No epithelial reaction
No secondary placenta	Secondary placenta	No secondary placenta
Trophoblast:		
Boundary between maternal and fetal tissues very irregular	Boundary between maternal and fetal tissues straight	Boundary between maternal and fetal tissues straight
Penetration of inner third of myometrium by trophoblast	No penetration of myometrium by trophoblast	No penetration of myometrium by trophoblast
Trophoblastic wandering cells prominent	No trophoblastic wandering cells	No trophoblastic wandering cells
Arteries:		
Intravascular cells later, peak at week 12	Intravascular cells very early, day 17	Intravascular cells very early, day 16
Artery wall replaced by trophoblast migrating through lumen and stroma	Artery wall replaced by trophoblast migrating through lumen only	Artery wall replaced by trophoblast migrating through lumen only
Little elastic tissue beyond myoendometrial junction	Elastic tissue extending halfway up endometrium	Elastic tissue extends well into endometrium
Multiple openings to intervillous space from single stems common	Multiple openings to intervillous space from single stems infrequent	Multiple openings to intervillous space from single stems common

*Reproduced with slight modification from Houston, M. L.: Am. J. Anat. **126:**1-29, 1969.

cells within the maternal capillaries and arteries communicating with the intervillous space. Sex chromatin studies indicate that these cells are cytotrophoblast.[6] Electron microscopy has provided even stronger evidence for this conclusion.[16] The natural history of these cells can be divided into three phases according to their relation to the vessels with which they are associated: (1) the intraluminal phase, in which the cytotrophoblastic cells are found within the lumen of the vessel, (2) the invasive phase, during which the cells penetrate the vessel wall, and (3) the intramural phase, when the cells constitute the wall of the vessel.

1. The intraluminal phase. Cytotrophoblastic cells be-

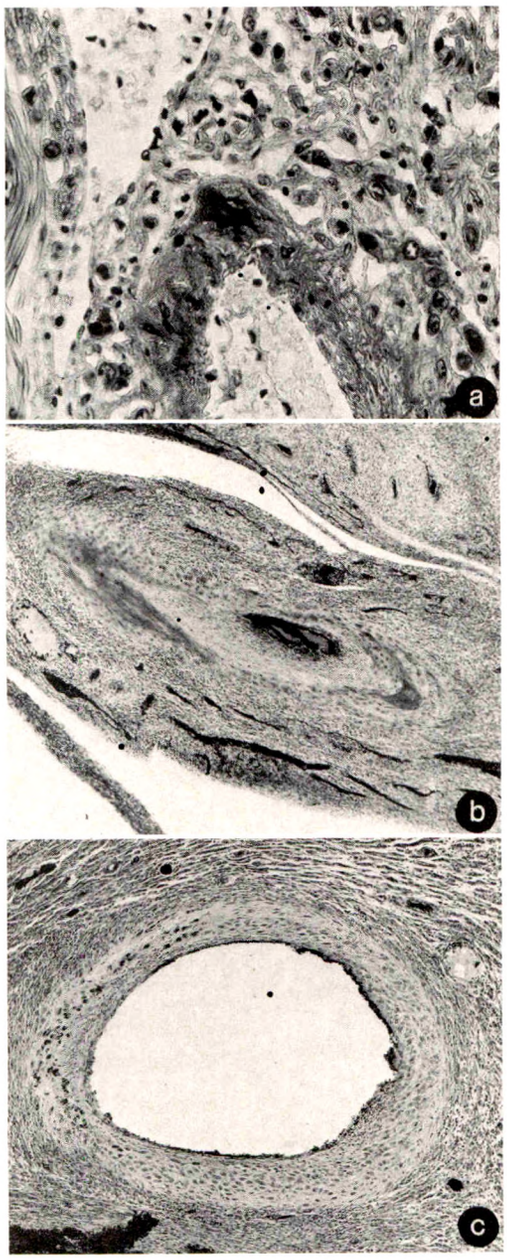


Fig. 4. Photomicrographs demonstrating arterial wall replacement by trophoblast in (*a*) the human subject, (Carnegie No. 10118, ×400) day 116—note uninvolved wall of vein at upper left and trophoblastic giant cells in stroma; (*b*) the rhesus monkey (Carnegie No. C-679, ×60), day 102; (*c*) the baboon (No. A67-98, ×60), day 144.

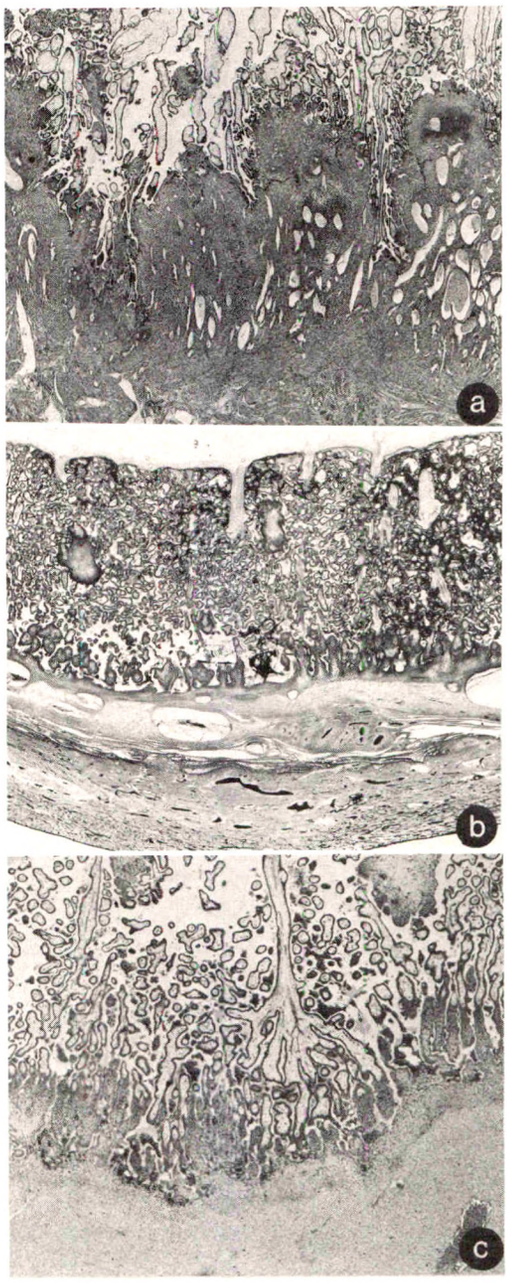


Fig. 5. Photomicrographs of the maternal-fetal junction in (*a*) the human subject (Carnegie No. 10129, ×10), very irregular, day 61; (*b*) the rhesus monkey (Carnegie No. C-629, ×3) day 53; and (*c*) the baboon (No. A65-103, ×40), day 47. Line of junction is smooth in monkey and baboon.

gin to migrate into the lumina of the maternal blood vessels as soon as the invading blastocyst encounters and "taps" the subepithelial capillary network (Fig. 3). These cells migrate along the walls of the expanded capillaries in sheets of cells, one to four layers thick, giving, in longitudinal section, an appearance which has been likened to wax dripping down the side of a candle. When the migrating cells reach the level of the spiral arteries they accumulate to the point of almost occluding the arterial lumina. In the human subject, migration proceeds into the deeper portions of the arteries, even involving myometrial segments. However, penetration beyond the myoendometrial junction has not been observed in either the rhesus monkey or

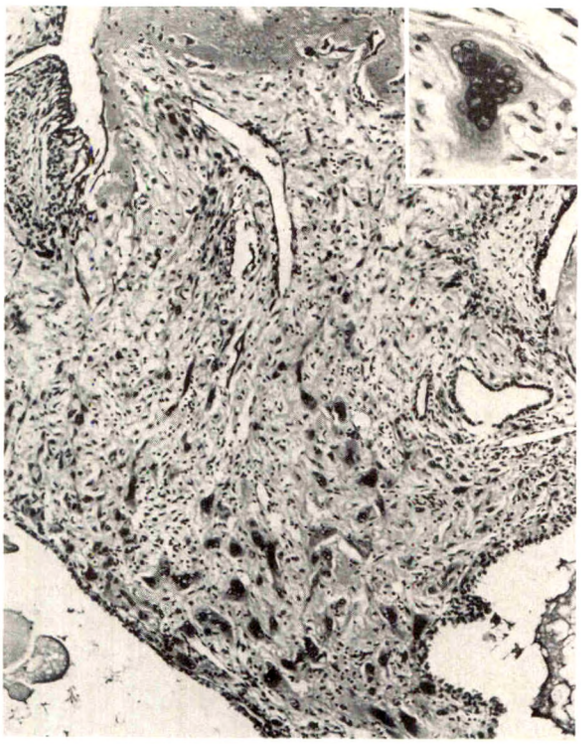


Fig. 6. Photomicrographs of trophoblastic giant cells in stroma of the human subject (U. of Va., No. U18-4, ×45). Inset shows cells at higher power.

the baboon. It must be noted that the phenomena described occur only in the arteries which communicate with the intervillous space and do not occur in veins at all.

Although the natural history and appearance of this intraluminal phase is basically similar in the three species there is a wide difference in the time of occurrence. In the human being, the cells can be observed within the arterial lumen early in gestation but they do not reach their peak of proliferation until as late as the twelfth week. In monkey and baboon the peak is reached very early and there is distinct diminution by the end of the second month of gestation.

2. *The invasive phase.* During invasion of the lumina of the spiral arteries, the cytotrophoblastic cells strip away the endothelium. The next step is invasion of the vessel wall. The tunica media becomes disorganized and cytotrophoblast becomes incorporated into the vessel wall, eventually forming the primary element of its structure.

3. *The intramural phase.* During this phase the cytotrophoblastic cells disappear from the lumina (Fig. 4). The endothelium of the vessels is regenerated from deeper regions not reached by the destructive action of the trophoblast. The wall continues to consist preponderantly of cytotrophoblast with which is associated abundant fibrinoid. Thus, maternal blood traverses the maternal tissue on its way to the placenta through vessels with an inner lining of maternal endothelium but with walls composed primarily of trophoblast.

Although the intrusion of the trophoblast into the maternal arteries is similar in the three species, there is diversity in the degree of invasion into the connective tissue. In human subjects, the line of trophoblastic penetration is extremely irregular, forming a jagged maternal-fetal junction (Fig. 5, *a*). In addition, the connective tissue stroma is heavily infiltrated with large, sometimes multinucleated, trophoblastic cells extending completely through the endometrium and frequently into the myometrium (Fig. 6). There is some evidence that these cells may invade the arterial walls and participate in their replacement. In the monkey and baboon, on the other hand, the trophoblast appears "less invasive" than in man in that (1) the implantation remains superficial, (2) the maternal-fetal junction occurs along a very regular cresentic line (Fig. 5, *b* and *c*), and (3) there is no penetration of the stroma by cells detached from the trophoblastic shell (Fig. 2, *b* and *c*).

In human patients, the trophoblast may even undergo malignant transformation, invading both locally and systemically as choriocarcinoma.

Comment

In the past it has been postulated that the decidua is responsible for restricting trophoblastic penetration of the endometrium. [3, 4, 14] Comparison between the amount of decidua formed in human, monkey, and baboon endometria and correlation of these amounts with the extent of trophoblastic invasion in the three species casts doubt upon the validity of this assumption. As is shown in Table I, it is the human subject that has the most pronounced decidua yet exhibits the greatest trophoblastic penetration. Where there is at best minimal decidualization, the trophoblastic invasion is least.

The barrier effect of the monkey's epithelial plaque has also been invoked as protective[13] but the absence of a plaque in the baboon rules out this assumption as well. The plaque in the monkey might restrict the trophoblast at first and be the effective cause of superficial implantation in the monkey, but what, one must ask, restrains the trophoblast when the plaque disappears in the third week of rhesus gestation or never forms at all as in the baboon?

The difference in the degree of fetal-maternal interaction is manifested not only by the depth of initial

implantation, as noted above, but later in pregnancy by the nature of the placental base. The jagged line of the maternal-fetal junction indicates a greater aggressiveness of the trophoblast in human beings than in the two nonhuman primate species. This is also suggested by the invasion of the human endometrial stroma, and even the myometrium, by wandering trophoblastic giant cells. The extreme rarity of choriocarcinoma in rhesus monkey and baboon may be cited as further evidence of a lower invasive potential of the trophoblast of these animals vis a vis that of human beings.

The entry of trophoblast into the eroded tips of endometrial spiral arteries is effected in similar fashion in all three species and the subsequent proximal spread is closely similar, in both mechanism and amount. Although the replacement of the vessel wall by trophoblast follows a parallel course in all the species, it is most extensive in human subjects, resulting in greater weakening of the vascular wall. This in turn occasions more pronounced dilation of the human uteroplacental arteries. In the studies of Brosens and associates [1, 2] variation in the amount of wall replacement is correlated with the occurrence of preeclampsia and eclampsia, conditions not known to occur in monkey or baboon.

The present comparative study rather dramatically illustrates the necessity for exercising caution in transferring observations made upon a single species to other even closely related species.

The courtesy of the Department of Embryology, Carnegie Institition of Washington, and of the Department of Obstetrics and Gynecology, University of Virginia School of Medicine, in permitting reproduction of photographs of material in their collections is gratefully acknowledged.

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CURRENT INVESTIGATION

This section offers prompt first announcement of new observations or discoveries. Articles should be limited to 1,500 words and six references. Illustrations or additional references require a proportionate reduction in total words.

Fetal blood sampling in midtrimester pregnancies

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YUET W. KAN, M.D.
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Sonographically directed placental aspiration has been investigated as a method for obtaining fetal erythrocytes. The technique is generally successful and the fetal red cells are generally usable for the prenatal diagnosis of the hemoglobinopathies.

THE PRENATAL diagnosis of genetic defects has been a major step forward for both obstetrics and human genetics. Amniotic fluid cells have been used to obtain information about the fetus, and the information available has been limited to that normally expressed in these cells. The prenatal diagnosis of a hemoglobinopathy or of a red cell enzymatic deficiency requires the ability to obtain fetal red cells. This has been attempted with the use of fetoscopy-directed aspiration[1, 2] and, in the case of an anterior placenta demonstrated by ultrasound, "blind" aspiration.[3] We have sought to define a method for obtaining fetal erythrocytes in a mixed fetal-maternal sample by sonographically directed placental aspiration regardless of placental location.

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Supported in part by grants from the National Foundation–March of Dimes, National Institutes of Health, and the John A. Hartford Foundation.

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Methods

The 28 patients in this study either were having elective pregnancy termination for socioeconomic indications or because of a chromosomally abnormal fetus or were referred to our program for prenatal diagnosis of a hemoglobinopathy. All patients gave expressed informed consent for the procedure as per a protocol approved by the University of California San Francisco Committee on Human Experimentation.

The patients undergoing pregnancy terminations had a sonographic placental localization with a full bladder and again after voiding. Bladder emptying was found to move the placental site 3 to 5 cm. caudally. In the case of a posterior placenta, the 20 gauge, 3 ½ inch, disposable spinal needle was introduced into the amniotic sac. Fluid aspiration and needle advancement were alternated until the needle bevel was against the fetal surface of the placental plate as indicated by a lack

Table I. Fetal blood samples obtained by placental aspiration

Fetal cells (%)	*No.*	*% of Total*
0-1	6	21.4
2-4	2	7.1
5-10	4	14.3
15-25	3	10.7
75-100	13	46.5
	28	100.0

of amniotic fluid return. The needle was inserted 2 to 4 mm. further for blood aspiration. A series of five or six samples was taken as the needle was withdrawn back into the amniotic sac, and a sample of the amniotic fluid in the immediate area of the placental puncture was included. One or two insertions into the placenta provided the 5 or 6 samples which need contain only 50 to 100 μl of blood mixed in amniotic fluid. A set of 5 or 6 samples was considered as one attempt at placental aspiration.

In the case of an anterior placenta, the placenta was traversed by the 20 gauge needle until amniotic fluid was obtained. The procedure was similar, but the needle was withdrawn until it reached the placental plate and then was withdrawn 2 to 4 mm. further, into the fetal surface of the placenta.

Women having a therapeutic abortion had only one attempt at placental aspiration, and then 40 mg. of prostaglandin $F_2\alpha$ was instilled in the amniotic sac to induce the abortion. Patients referred for the prenatal diagnosis of a hemoglobinopathy had multiple attempts, if necessary, to obtain fetal red cells.

The presence of fetal erythrocytes was determined by analysis of red cell volume by a Coulter counter since the fetal cell volume is larger than that of adult cells.[4] In the last half of the study, the percentage of fetal cells was verified by Kleihauer-Betke stains of smears from the same samples.

Results

The percentage of fetal erythrocytes in placental aspirates is given in Table I. Samples from the nine posterior placentas had 2 to 100 per cent fetal cells, and in no case was a second attempt necessary. Eleven of 16 (69 per cent) anterior placentas yielded samples of 2 per cent or more fetal red cells, but in two patients this required three attempts each. Two of three lateral placentas provided samples with 22 and 75 per cent fetal cells.

Of the eight patients referred for prenatal diagnosis of a hemoglobinopathy, four fetuses were found to be homozygously affected, the pregnancies were terminated, and the prenatal diagnosis was confirmed.

Comment

For prenatal diagnosis of the hemoglobinopathies, a mixture of fetal and maternal blood can be used and, by concentrating fetal cells with differential agglutination with the use of anti-i serum, as little as 2 per cent fetal erythrocytes may be adequate.[3] With that criterion, 100 per cent of the posterior placentas, 69 per cent of the anterior placentas, and two of three lateral placentas were successfully sampled by sonographically directed placental aspiration. This compares favorably with the one other report of placental aspiration of anterior placentas.[3]

The largest reported series of patients in whom fetal blood sampling was attempted under fetoscopic visualization of the placenta claimed success in 19 of 20 instances with a posterior placenta and 4 of 6 instances with an anterior placenta.[2] However, no definition of success was given in this paper. If the two methods have essentially similar success rates, which is the better method for obtaining fetal erythrocytes will depend on which is safer. There is insufficient experience with either method at this time to make any estimates of safety; however, use of the fetoscope in the presence of an anterior placenta is of considerable concern. Regardless of technique, we agree with Hobbins and Mahoney[2] that fetal blood sampling should be done after 18 weeks' gestation, since with increasing gestation a given blood loss represents a smaller proportion of the fetal blood volume.

Although we have employed this technique to successfully diagnose β-thalassemia and sickle cell anemia in utero,[5, 6] the method's shortcomings should be noted. The only case of amnionitis followed two successive days of attempting to obtain fetal blood. There probably was a break in the sterile technique on the second day, and the amniotic fluid containing maternal blood from the first day's efforts provided an excellent culture medium. We now feel that if initial attempts at fetal blood sampling are unsuccessful one should wait 7 to 10 days before a second set of attempts. This will allow for reabsorption of blood in the amniotic fluid. We are concerned also with the question of fetal-maternal transfusion and isoimmunization and accordingly have begun an ongoing project of estimating the fetal-maternal transfusion induced by placental aspiration.

We conclude that fetal erythrocytes for genetic studies can be obtained by sonographically directed

placental aspiration. This technique is simple, and the potential benefits of fetal blood sampling are sufficient to warrant further investigations of its effectiveness and safety.

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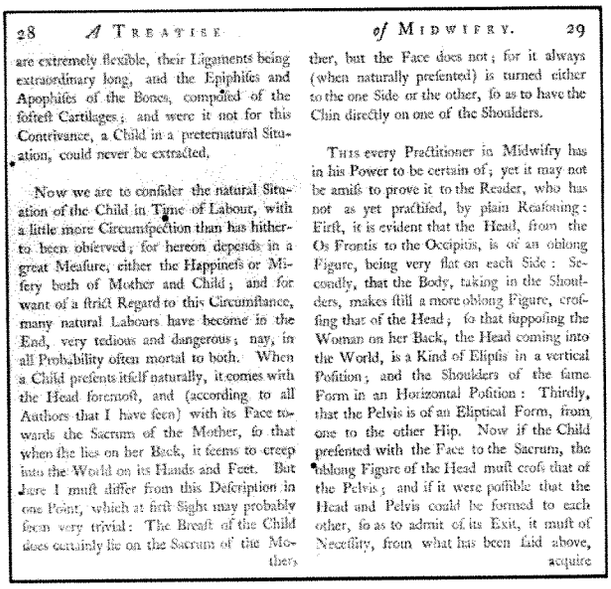
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Classic pages in
OBSTETRICS AND GYNECOLOGY
Lawrence D. Longo, M.D.

A treatise of midwifry. In three parts

SIR FIELDING OULD

Dublin, O. Nelson & C. Connor, 1742



28 *A* TREATISE *of* MIDWIFRY. 29

are extremely flexible, their Ligaments being extraordinary long, and the Epiphiſes and Apophiſes of the Bones, compoſed of the ſofteſt Cartilages; and were it not for this Contrivance, a Child in a preternatural Situation, could never be extracted.

Now we are to conſider the natural Situation of the Child in Time of Labour, with a little more Circumſpection than has hitherto been obſerved; for hereon depends in a great Meaſure, either the Happineſs or Miſery both of Mother and Child; and for want of a ſtrict Regard to this Circumſtance, many natural Labours have become in the End, very tedious and dangerous; nay, in all Probability often mortal to both. When a Child preſents itſelf naturally, it comes with the Head foremoſt, and (according to all Authors that I have ſeen) with its Face towards the Sacrum of the Mother, ſo that when ſhe lies on her Back, it ſeems to creep into the World on its Hands and Feet. But here I muſt differ from this Deſcription in one Point, which at firſt Sight may probably ſeem very trivial: The Breaſt of the Child does certainly lie on the Sacrum of the Mother, but the Face does not; for it always (when naturally preſented) is turned either to the one Side or the other, ſo as to have the Chin directly on one of the Shoulders.

THIS every Practitioner in Midwifry has in his Power to be certain of; yet it may not be amiſs to prove it to the Reader, who has not as yet practiſed, by plain Reaſoning: Firſt, it is evident that the Head, from the Os Frontis to the Occipitis, is of an oblong Figure, being very flat on each Side: Secondly, that the Body, taking in the Shoulders, makes ſtill a more oblong Figure, croſſing that of the Head; ſo that ſuppoſing the Woman on her Back, the Head coming into the World, is a Kind of Eliptis in a vertical Poſition; and the Shoulders of the ſame Form in an Horizontal Poſition: Thirdly, that the Pelvis is of an Eliptical Form, from one to the other Hip. Now if the Child preſented with the Face to the Sacrum, the oblong Figure of the Head muſt croſs that of the Pelvis; and if it were poſſible that the Head and Pelvis could be formed to each other, ſo as to admit of its Exit, it muſt of Neceſſity, from what has been ſaid above, acquire

The early eighteenth century was an age when deliveries were performed mainly by unskilled midwives and most physicians were equally ignorant of the process of childbirth. Ould's treatise was the first textbook of any importance in English, the first to consider the mechanism of labor and, over all, a veritable mine of good sense. The first part described the pelvis, female genitals, the fetus, and normal labor. It was formerly held that the child's face pointed toward the sacrum during its transit through the pelvis. In contrast, Ould asserted that the child faced obliquely to one side or the other (see left). He regarded labor as a mechanical process, stressing that since both the head of the child and maternal pelvis were elliptical the smaller ellipse could pass through the larger only if they were in the same axis. He advocated delivery on the side, opiates in difficult or tedious labours, and delivery of the second twin shortly after that of the first, by version and extraction if necessary, and cautioned against overhasty extraction of the placenta. Part II dealt with abnormal deliveries not requiring instruments. He opposed the view of Deventer and Mauriceau that "obliquity" of the uterus was a common cause of difficult labor. In breech delivery, he emphasized the importance of bringing down the arms. "In the directions for bringing the child forth by the feet, (Deventer) adviseth the greatest piece of cruelty that can be imagined; namely, to leave the hands to come out together with the head; the absurdity of which practice is obvious to any person who ever delivered one woman, even in a natural labour; indeed if he had advised this practice in a miscarriage, after six months pregnancy, and where there is an immoderate rate of flux of blood; then this method, for expedition, might be commendable." Sir Fielding delivered the breech infant with his finger in the child's mouth and hands on its shoulders. In the third section on instrumental delivery, he was one of the earliest advocates of episiotomy but was intolerant of cesarean section. A leading obstetrician of his time, Ould was master of the Dublin Lying-In (later known as the Rotunda) Hospital.

COMMUNICATIONS IN BRIEF

This section is suitable for reporting cases, results of therapeutic trials, and descriptions of new procedures or instruments. Reports should be limited to seven hundred words and two references. Use of an illustration or table requires a proportionate reduction in total words.

An unusual complication of intrapartum fetal monitoring

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A CASE OF second-degree scalp burn caused by a spiral electrode, during intrapartum fetal monitoring, is reported. Such a serious complication can occur without showing changes in the fetal heart rate. It is emphasized that this complication should be kept in mind when using an internal monitoring system.

Interest in the evaluation of the fetus by electrocardiography has increased since Hon and Hess[1] and Caldeyro-Barcia and associates[2] pioneered the measurement of beat-to-beat variation in the fetal heart by electrocardiogram recording. Tracings of the recordings are generally of good quality when the internal monitoring system is used.

Complications such as local cellulitis and hemorrhage associated with the application of scalp electrodes have been recognized. The following case is reported because of a hitherto unreported complication.

A 20-year-old primigravida had labor induced by dilute oxytocin infusion due to pre-eclamptic toxemia at term gestation.

The membranes were ruptured with an amniotomy hook during labor, when the cervix was 3 cm. dilated. A spiral scalp electrode, which had been sterilized by keeping it soaked in 2 per cent aqueous activated dialdehyde solution (Cidex) for 12 hours, was washed in normal saline and then applied to the fetal scalp with the help of a sterilized electrode director. The application of the electrode was easy as the head was at the level of the ischial spines.

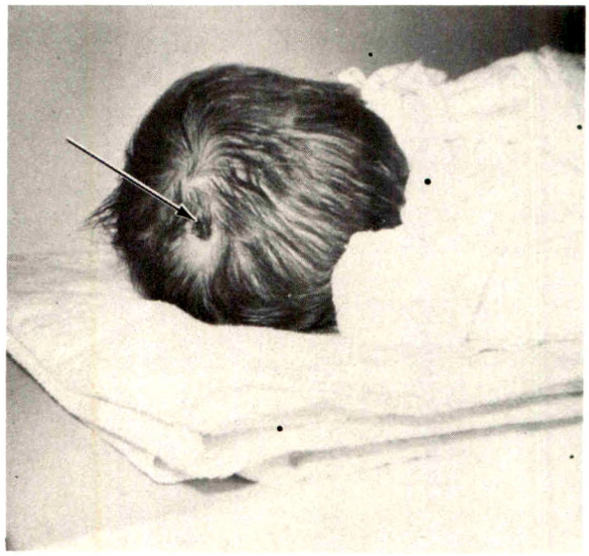


Fig. 1. Head of the newborn infant. The arrow demonstrates the area involved in the burn.

Leads from the scalp electrode were connected to the machine for direct monitoring of the fetal heart. The connections of the electrode leads and machine plug were properly secured. The leads were kept separated by means of rolls of gauze and then placed over the upper part of the lateral aspect of the thigh by means of an adhesive plaster.

Direct fetal heart rate recording after the application of the spiral electrode did not show any deceleration patterns.

The policy of our obstetric unit being that all high-risk pregnancies are continuously monitored during labor, in this case the uterine contractions and the fetal heart rate were continuously recorded and there was no evidence of fetal heart deceleration about 5 minutes before the delivery.

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Am. J. Obstet. Gynecol.

A healthy and mature female baby was delivered without any difficulty. The baby had an Apgar score of 8 at 1 minute after the birth.

Soon after the birth of the head of the baby the monitoring equipment was switched off and the spiral electrode was detached.

A dark and coarse area about 1 cm. in diameter was noticed at the site where the scalp electrode had been attached during monitoring. On thorough examination of this area, a second-degree burn of the scalp was confirmed. Fig. 1 demonstrates the burn on the left occipitoparietal area of the head of the baby. An extensive search was made for any fault in the connections of the electrode leads and the monitoring equipment, but no cause could be found at that time.

The burn was treated and the baby was closely observed jointly by the pediatrician and ourselves. The baby recovered completely. She is 14 months old now. Apart from a hairless area at the site of the burn she shows no other abnormality.

Minor complications such as local cellulitis, scalp abscess, and hemorrhage from the site of the electrode have been previously reported.[3] To the best of our knowledge this is the first case of scalp burn caused by the spiral electrode used for direct fetal heart monitoring.

Although faint yellow staining of the skin in animals due to local effect of 2 per cent activated glutaraldehyde solution has been described,[4] this was not considered to be the cause in this case. The electrode had been thoroughly washed with normal saline before being applied to the fetal head.

Another possibility considered was short circuit in the monitoring equipment, but the manufacturers of the equipment checked this and reported their machine in perfect condition.

The mechanisms involved in producing this serious complication have remained unclear up until this time but, to our great relief, that monitoring equipment has been replaced by a new machine by the manufacturers.

As the internal method of fetal monitoring is more reliable than the external method,[5] the application of scalp electrodes can be expected to increase in obstetric units. We suggest that such a complication be kept in mind while using them for this purpose.

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Incidental tubal ligation with Falope Ring Applicator at the time of cholecystectomy

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Incidental appendectomy has been done in conjunction with cholecystectomy, although some feel this should not be done because of the possibility of added morbidity. Bilateral partial salpingectomy has also been done with cholecystectomy; however, this is frequently a cumbersome procedure because of exposure difficulties in the pelvis.

Although the use of the laparoscopic tubal instruments would help in reaching the pelvic cavity, the use of thermoenergy in these cramped quarters could be hazardous. A recent refinement by Yoon and associates[1] in laparoscopic sterilization with the use of a Falope Ring Applicator obviates the use of thermoenergy in the pelvis.

From the upper abdominal incision, the tubes can be visualized, aided by a uterine elevator, and the Falope Ring bands can be placed over the tented portions with ease. This can be accomplished with only a few minutes added to the operative time and little likelihood of increased morbidity.

A 37-year-old woman, gravida 2, para 2, was admitted to the hospital with right upper quadrant pain; the diagnosis was cholelithiasis. It was felt that a cholecystectomy was necessary, and the patient requested tubal sterilization at the same time.

She wished no further children and was an ideal candidate for tubal sterilization. She had no significant gynecologic complaints and at the time was using a CU-7 intrauterine device for contraception.

A vertical midline upper abdominal incision was made, and the cholecystectomy proceeded without difficulty. At the end of this procedure, with the aid of Semm's uterine cannula, the tubes were easily visualized bilaterally.

From the cholecystectomy incision, the tubes were picked up with the Falope Ring applicator, and the silicone rubber bands were placed over the tented portions. Postoperative recovery from the operative procedures was uneventful.

It is suggested that elective tubal sterilization with the silicone rubber band technique can easily be done at the time of an upper abdominal operation without adding substantial operative time or morbidity.

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Placenta previa in association with an incompetent cervix

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THE PATIENT, a 23-year-old primagravid nurse, was seen initially at 6 weeks' gestation. She had a 2 cm. sessile endocervical polyp.

At 15 weeks' gestation sharp cramps and vaginal bleeding occurred and the cervix was found to be 2 cm. dilated with membranes herniating into the cervical canal. External cerclage with No. 2 silk as a purse string was of transient benefit and she aborted a female fetus weighing 150 grams 3 days later.

Hysterosalpingogram 8 weeks after this event ruled out bicornuate uterus. Her endocervical polyp had undergone spontanous resolution.

She conceived again 10 weeks after the hysterosalpinogram and cerclage was suggested for 13 weeks. She bled for 1 hour at 8 weeks' gestation. Delalutin 250 mg. was started and suggested at intervals of 2 weeks for five doses along with the usual prenatal supplements of iron, folic acid, and vitamin C. She had slight bleeding at 10 and 12 weeks, but the cervix remained closed and the fetal heart tones were recorded as good with the MiniDop Ultrasonic Device.

Effacement was evident, however, at 13 weeks and a Mersilene ribbon was inserted under the vaginal skin at the level of the internal cervical os. The procedure was uneventful.

Ten days later she commenced heavy bleeding through the cervical canal and because abruptio placentae was possible the Mersilene ribbon was removed under local anesthesia. The fetal heart tones remained good and the uterine muscle quiescent, despite two episodes of heavy bleeding. The hematocrit fell from 38 to 29 percent. The coagulation profile remained normal. After cessation of flow she continued bedrest for 2 weeks. The cervix remained effaced but not dilated more than 1 cm. An external purse-string cerclage with No. 2 silk was then inserted. There was no subsequent bleeding. She remained at bedrest except for bathroom rituals and short nonstrenuous outings. Ultrasonography at 25 weeks' gestation revealed a posterior placenta previa. At 36 weeks after conception a low segment transverse cesarian section was performed with delivery of a viable male infant weighing 2,863 grams. A placenta previa involving the posterior wall and the entire cervical os was confirmed.

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Unusual twin pregnancy in a double uterus

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THE PURPOSE OF this report is to present an unusual case of pregnancies in both horns of a double uterus, one horn of which had no communication to the vagina or the other horn. When the patient requested an abortion for the second time in 2½ months, both sonography and hysterography were of significance in arriving at the correct diagnosis.

The patient was a 25-year-old, married, white woman, gravida 3, para 1, with one abortion. The last menstrual period was on October 12, 1973. When she realized that she was pregnant, she went to an Abortion Clinic on December 5, requesting interruption of the pregnancy. At the examination, the uterus was estimated to be of seven weeks' size, compatible with the menstrual history. The following day, suction curettage was performed; the uterine cavity measured 10 cm. The pathology report showed decidua and chorionic villi. She had not resumed sexual relations when, on January 19, 1974, she was examined again. The uterus then was enlarged, corresponding to a pregnancy of 12 to 14 weeks. The patient was advised to have a saline abortion. After delay of a month, she presented in our Family Planning Clinic for the first time on February 19, 1974, requesting an abortion. The uterus was now 17 to 18 weeks by size. She was admitted for a midtrimester abortion on February 25, and induction was initiated with vaginal suppositories of prostaglandin $F_{2\alpha}$, 10 mg. every 2 to 4 hours. This induced uterine cramps, but the cervix remained closed; therefore, the medication was discontinued.

A sonogram was then obtained which was interpreted as either a 19 week pregnancy in one cornu of a bicornuate uterus or an abdominal pregnancy. Subsequently, hysterography was performed, demonstrating a sickle-shaped uterine cavity, pointing to the right, and one Fallopian tube on the right side. There was no filling at all on the left side, although the mass considered as the pregnancy was palpated in the left side of the pelvis on examination under anesthesia (Figs. 1 and 2).

On February 27, an exploratory laparotomy was performed. There was a double uterus, consisting of a smaller right horn with a cervix at the top of the vagina, and a larger, obviously pregnant, left horn with no connection to the right horn or the vagina except for a solid fibrous band, almost 1 cm. thick, to the cervix of the right horn (Fig. 3). On both sides, normal tubes and ovaries were seen, but the left kidney could not be palpated. The left uterine vessels ran parallel to

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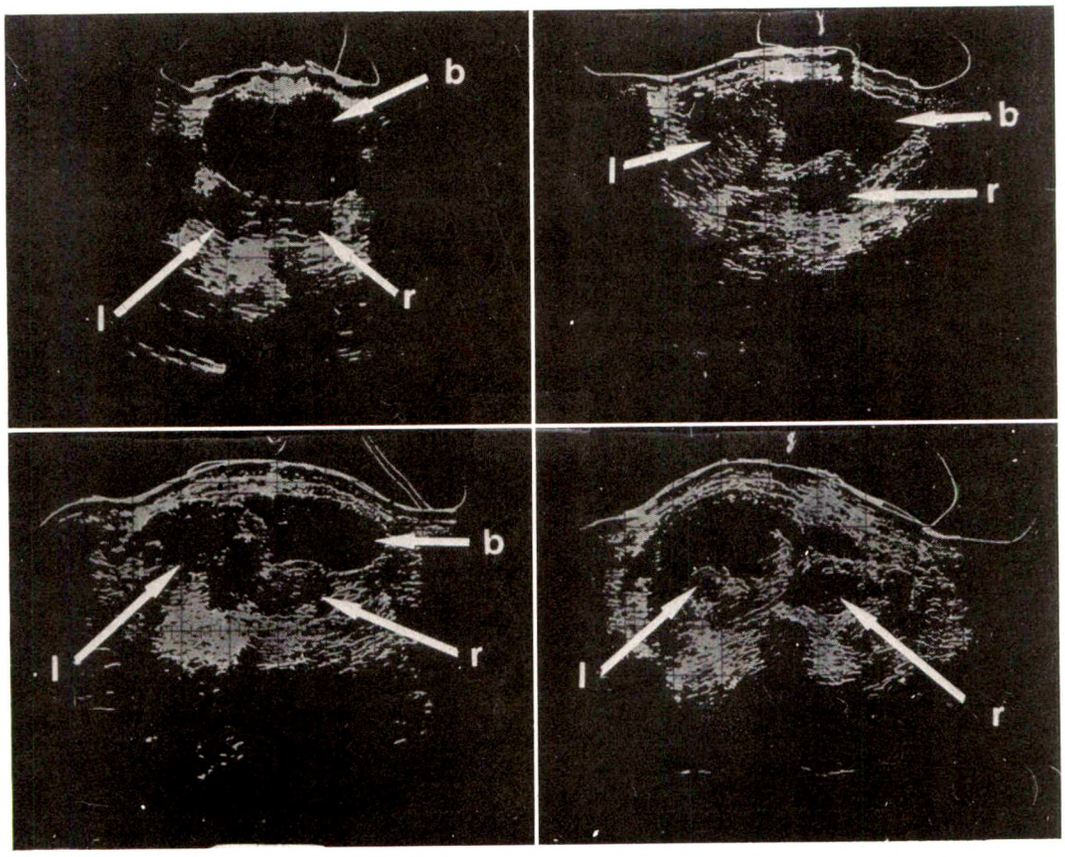


Fig. 1. Transverse sonograms of the right cornu and left horn with pregnancy echoes inside. *r* = Right horn; *l* = left horn; *b* = bladder.

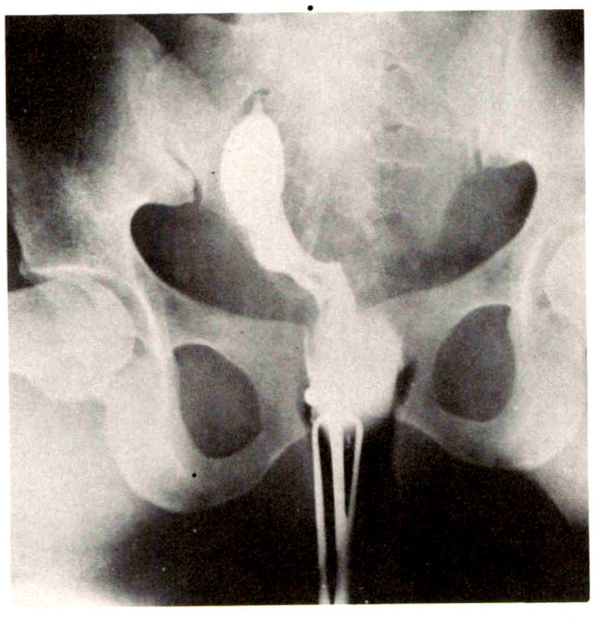


Fig. 2. Hysterogram of the right uterine horn laterally displaced by the left pregnant rudimentary blind horn.

the fibrous band. It was decided to resect the left horn with the pregnancy but to preserve the left ovary.

The pathology report indicated a fragment of uterus measuring 8 by 6.5 by 5.5 cm. and weighing 90 grams. The external surface exhibited a fragment of Fallopian tube 2 cm. in length. There was a female fetus weighing 130 grams and measuring 17 cm. in crown-heel length. The final diagnosis was ectopic pregnancy in the blind horn of the uterus.

A postoperative intravenous pyelogram confirmed the absence of the left kidney.

This unusual case raises several interesting questions. Since the blind left horn could accomodate a pregnancy, it must have had functional endometrium. What happened to the menstrual blood between the menarche and the pregnancy in this 25-year-old woman? Why did she not develop a hematoma on this side?

The ovum for the pregnancy on the left side could have originated from either ovary, but the fertilizing spermatozoa must have entered the abdominal cavity through the right uterine horn and the right tube. Presumably, fertilization took place in the cul-de-sac, after which the fertilized ovum must have been picked

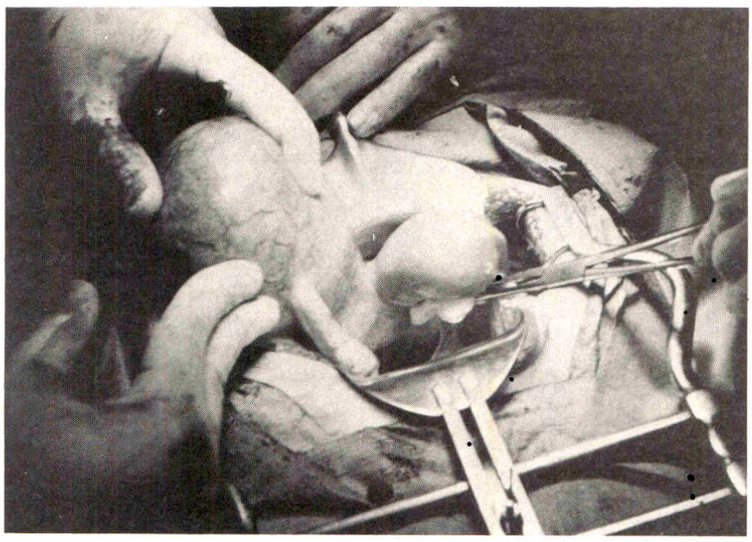


Fig. 3. Pregnancy in left rudimentary horn at laparotomy (supported by the operator's hands). Right uterine cornu elevated by sponge stick.

up by the left tube. If the suction procedure done elsewhere on December 5 did remove gestational products and not just decidua, two ova must have been fertilized simultaneously, one fertilized ovum subsequently implanting in each horn of the double uterus.

The pregnancy in the blind horn had a considerable danger potential.[1] Fortunately, the midtrimester abortion was induced with prostaglandin, permitting discontinuation of induction when no progress was observed. Perseverance with prostaglandin administration or intra-amniotic injection of hypertonic saline could presumably have led to rupture of the horn. The same could have happened if the pregnancy had been allowed to continue.[2]

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Fatal maternal disseminated coccidioidomycosis in a nonendemic area

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APPROXIMATELY 65 patients with coccidioidomycosis in pregnancy have been reported. Of these, 37 cases were disseminated, and 29 patients died. All disseminated cases were from the endemic areas.[2, 4] The following report is presented to reinforce the need for an awareness of disease states outside one's own locale, especially in view of our highly mobile population.

The patient was a gravida 3, para 1, 27-year-old, Caucasian woman. She had briefly visited Phoenix, Arizona, at 18 weeks' gestation. The pregnancy was uncomplicated until 27 weeks' gestation when she complained of "blurred vision" and a "headache." Two weeks later, she described a "very throbbing headache," a "halo effect" in the left eye, "hot flashes," and symptoms of an upper respiratory tract infection with sinusitis and a dry cough. Treatment with penicillin G, Tuss-Ornade,* and Tylenol with codeine had no effect. One week later, she was admitted to the hospital with a temperature of 103.8° F and expressive aphasia. The working diagnosis was a brain abscess.

Chest x-ray, skull films, and brain scan were negative except for "clouding" of the left frontal sinus. High doses of penicillin and chloramphenicol were used without apparent effect. Temperature fluctuated widely. When afebrile, the sensorium and speech were clear; when febrile, she was confused and semiconscious.

On the fifth hospital day (32 weeks' gestation), she developed labored respiration, and chest x-ray showed striking changes from five days earlier, consisting of disseminated, interstitial, bilateral infiltrates with a fine nodular pattern. The patient's liver and spleen had enlarged since the admission examination.

Spontaneous labor commenced on the seventh hospital day, and she was delivered of a 4 pound 2 ounce vigorous female infant. The placenta is shown in Figs. 1 and 2. A frozen section of a placental infarct showed the endosporulating spherules of coccidioidomycosis.

The placenta weighted 359 grams. A number of tan, irregularly shaped infarcts were scattered throughout the soft spongy lacerated tissue. A section taken through the edge of

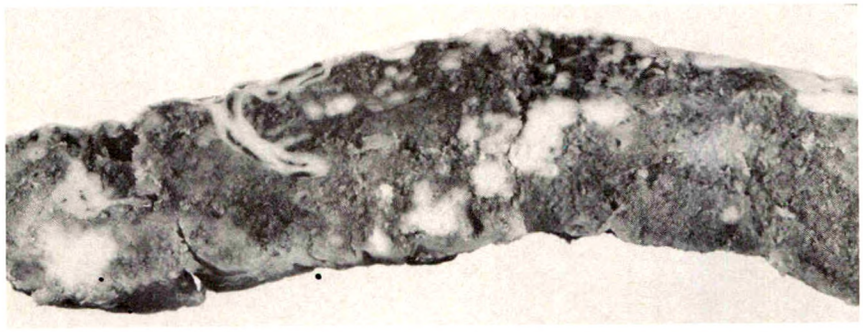


Fig. 1. Cut section of placenta showing the numerous poorly defined areas of necrosis.

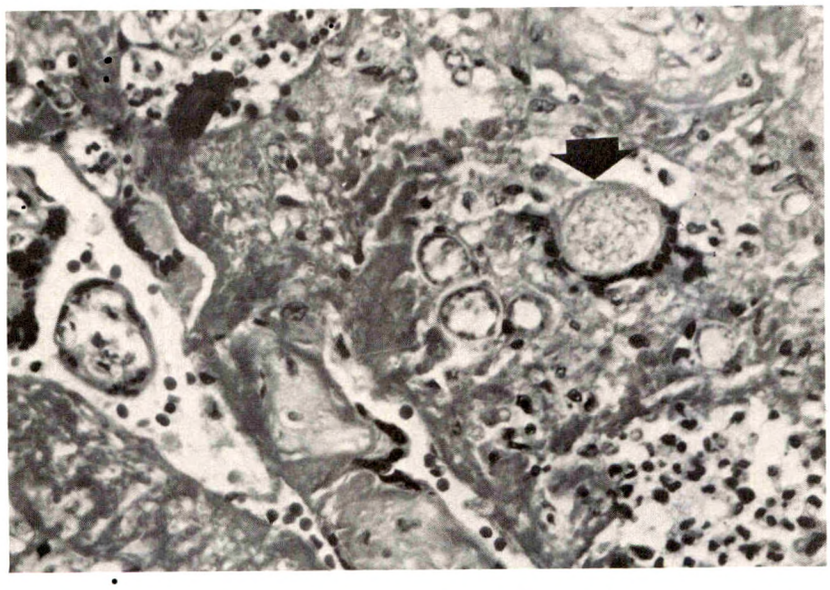


Fig. 2. Photomicrograph of placenta showing coccidioidomycosis. The arrow indicates the characteristic spherule filled with endospores.

one of the infarcts showed necrosis and acute inflammation and the presence of spherules of coccidioidomycosis. The placental tissue between these areas was not involved.

Complement-fixation titers drawn on the third postadmission day for coccidioidomycosis were 1:128. Cultures of maternal blood, sputum, spinal fluid, and vaginal secretions were all positive for coccidioidomycosis, but the patient died before these results were available.

Coccidioidomycosis is caused by the fungus *Coccidioides imitis*. It is indigenous in only a few areas in the New World, chiefly in the arid areas of the Southwestern United States, adjacent parts of Mexico, and the San Joaquin Valley of California. The arthrospores, which exist in the soil, are blown by the wind and infect the majority of those living in these areas. Symptoms appear in about 40 per cent of those infected; the others are identified only by skin testing.

Disseminated coccidioidomycosis is usually localized to the lung and simulates any common upper respiratory infection which clears with symptomatic treatment. Dissemination occurs in less than 0.2 percent of those infected. In tissue, the organism appears as thick-walled spherules, 30 to 60 μ in diameter, containing numerous rounded endospores. The tissue shows necrosis with infiltration by polymorphonuclear leukocytes; peripheral to this, in older lesions, is a granulomatous-type reaction. The tissue form of the fungus is not infectious; therefore, patients need not be placed in isolation. In disseminated cases, the complement-fixations test, precipitation test, or other immunologic tests should be positive. Diagnosis depends on demonstration of the fungus in sputum, cerebrospinal fluid, or tissue or a demonstrated rise in titer of serologic tests. Skin tests are frequently negative in disseminated disease. Eosinophilia of 5 to 8 per cent may be seen in peripheral blood.

The present case illustrates several facets of the disease in pregnancy:

1. Patients who develop primary coccidioidomycosis when pregnant are at extremely high risk. If un-

treated, approximately 90 per cent die.[3] Up to 50 per cent may be saved by early adequate treatment with amphotericin B.

2. Maternal genital tract involvement is rare.[6] Our patient apparently had coccidioidomycosis in the vaginal secretions.

3. Placental involvement has been reported but is rare.[1, 2, 6] The diagnosis in our patient was from placental involvement.

4. There have been only two cases of possible fetal involvement with the disease.[2, 4] Our patient also had no evidence of fetal coccidioidomycosis.

5. The 50 per cent reported fetal loss is from prematurity.[4] The infant in the reported case died from hyaline membrane disease secondary to prematurity.

6. Passive transfer of maternal complement-fixing antibodies were shown in the cord blood.[5]

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Primary adenocarcinoma of the vermiform appendix in a gynecologic patient

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Primary adenocarcinoma of the appendix is a rare clinical entity that is virtually never diagnosed preoperatively. Hesketh[1] showed that in 13 per cent of the female patients with this neoplasm the diagnosis was made because an incidental appendectomy accompanied a primary gynecologic procedure. During the past decade at The Walter Reed Army Medical Center 67 per cent of all primary appendiceal neoplasms in females were discovered on the gynecology service. Since the gynecologist is likely to encounter most appendiceal neoplasms in females, this case report and review are submitted.

J. P., a 26-year-old white woman, para 1-0-0-1, was admitted for evaluation of abdominal pain and ascites. The onset of symptoms occurred 3 weeks prior to admission.

The past medical history and the review of systems were essentially noncontributory.

Physical examination revealed a 5 foot 8 inch, 150 pound woman with stable vital signs. The examination was unremarkable except for abdominal and pelvic findings. The abdomen was markedly distended with ascitic fluid. On pelvic examination bilateral large adnexal masses and multiple cul-de-sac nodularities were found.

The hemogram, urinalysis, and serology were within normal limits. The blood chemistries were unremarkable except for lactic dehydrogenase of 515 mU. per milliliter, total protein of 4.7 grams, and albumin of 2.7 grams. The chest x-ray, excretory urogram, upper gastrointestinal series, and barium enema were negative. The paracentesis fluid yielded class V cytology.

On the fourth hospital day an exploratory coeliotomy was performed and 4,500 c.c. or ascitic fluid were removed. Both ovaries were involved with tumor and each measured 12 cm. in diameter. Multiple excrescenses existed on the peritoneum, small and large intestines, and their mesenteries. Metastases to four para-aortic and two left common iliac nodes were found.

A frozen section analysis of the ovary was suggestive of a Krukenberg tumor. Evaluation of the intestinal tract revealed a neoplasm that encompassed the distal half of the appendix.

A total abdominal hysterectomy with bilateral salpingo-oophorectomy, partial omentectomy, and appendectomy were performed. The postoperative course was uneventful. The permanent tissue slides were diagnostic of a primary colloid adenocarcinoma of the appendix. Management was discussed at a combined oncology-radiotherapy conference. Chemotherapy with 5-fluorouracil was selected. Despite this therapy rapid tumor growth occurred. Death occurred 13 months after the diagnosis was made.

Primary appendiceal neoplasms account for about 0.5 percent of all gastrointestinal tumors.[1]

Carcinoid, in contradistinction to adenocarcinoma, is the most common and least malignant tumor. On cross-section, it has a golden-yellow appearance.[1] A malignant mucocoele is usually characterized by a dilated appendix containing gelatinous material or by a ruptured appendix and pseudomyxoma peritonei.[2] This tumor, unlike adenocarcinoma, rarely spreads by the blood stream or by the lymphatics. Adenocarcinoma may be a slow growing in situ lesion[2] or it may have a rapid transcoelomic spread with peritoneal and ovarian deposits.[1] About 66 per cent of all cases present as some form of appendicitis or appendiceal abscess.[1]

Carcinoid, mucocoele, and adenocarcinoma in situ

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or reflecting the views of the Department of the Army or the Department of Defense.

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are best managed by simple appendectomy with wide excision; right hemicolectomy is indicated if nodal metastases occur or if there is extension to the line of resection.[1, 2] Frozen section is suggested when any appendiceal neoplasm is found so that prompt surgery could be performed if indicated.

A review of the English literature shows that 4 out of 53 female patients with advanced appendiceal adenocarcinoma had ovarian metastases. Future investigation may reveal the potential benefits of prophylactic bilateral oophorectomy.

It appears reasonable that all abdominal gynecologic procedures should include evaluation of the appendix and, where feasible, prophylactic appendectomy.

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CORRESPONDENCE

Squamous cell neoplastic change in patients with vaginal adenosis

To the Editors:

We feel we must take exception to the implication that there is a high incidence of potentially malignant squamous cell neoplastic change in patients with vaginal adenosis as stated in the article, "Squamous neoplasia of vagina related to DES syndrome," by William C. Fetherston, M.D., which appeared on page 176 of the May 15, 1975, issue of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY.

Dr. Fetherston stated that 6 of the 46 patients (13 percent) in his series had biopsy-proved squamous dysplasia while a seventh patient showed squamous carcinoma in situ. This is in contrast to our experience, summarized in an article recently accepted for publication in the *American Journal of Clinical Pathology*,[1] in which we found no case of true squamous cell dysplasia in a group of 88 women with vaginal adenosis. In evaluation of almost 80 additional women, we have encountered only one patient with squamous cell dysplasia of a mild to moderate degree. Likewise, in a recent article, Herbst and associates[2] reported finding only four cases of mild squamous dysplasia after cytologic examination of vaginal scrapings or aspirates in 110 DES-exposed subjects, and "This change was not confirmed in any by multiple punch biopsies."

The differing incidences of dysplasia found in Dr. Fetherston's series and in our own might be due to differences in histologic criteria for dysplasia. No criteria are given in Dr. Fetherston's article, but we reserve the term "dysplasia" for lesions showing distinct nuclear abnormalities, including hyperchromasia, nuclear pleomorphism, and crowding with loss of polarity. In our experience, many of the atypicalities noted colposcopically, such as white epithelium, mosaicism, etc., correlate histologically with squamous metaplasia, poorly glycogenated mucosa, or regenerative responses to inflammation. These patterns lack nuclear abnormalities and have no known premalignant potential. Figs. 6 and 8 in Dr. Fetherston's article seem to show active squamous metaplasia and basal cell reparative activity, respectively, while Figs. 7 and 9 lack sufficient detail to be adequately evaluated.

We agree that the potential for dysplastic and malignant squamous lesions exists in vaginal adenosis. However, one should not, without more evidence, overemphasize the potential danger. To do so could precipitate unnecessarily radical treatment for adenosis in these young women, treatment perhaps associated with medical and emotional problems.

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Reply to Drs. Burke and Antonioli

To the Editors:

The hard data emphasized in this presentation consists of colposcopically atypical squamous epithelium in the transformation zone of the patients exposed to diethylstilbestrol (DES). This atypical epithelium was present in 87 per cent of the patients followed. The slides of the squamous cell carcinoma in situ reported were reviewed by at least a dozen pathologists, all of whom agreed with the diagnosis without exception.

I also know of at least seven other squamous lesions in situ which, as yet, have not been reported, all of which appeared in atypical transformation zone squamous epithelium in patients exposed to DES.

It is true, as Drs. Burke and Antonioli have stated, that there is poor correlation histologically between the colposcopic findings and many of the biopsies. Correlation between these patterns and histology of the cervix is significantly better. This may be due to the relatively young age of this group of patients. We also have found considerable difficulty in interpreting many of the less severe changes histologically, but our criteria are essentially the same as those of Drs. Burke and Antonioli.

We also caution against overtreatment of any but the frankly malignant lesions. The dangers in overtreatment in this otherwise normal group of young women are obvious, and we do share the concern of Drs. Burke

and Antonioli. The main thrust of this paper was to present the high incidence of colposcopic abnormalities in transformation zone epithelium in these patients, and suggest that the best way of following these changes, until the ultimate significance is clear, would be with repetitive colposcopic examination.

This is obviously a preliminary report, since none of us has followed these patients for more than four years, and only time and continued observation will determine this ultimate significance.

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Bilateral renal agenesis

To the Editors:

M. le Docteur Pierre Dellenbach (AM. J. OBSTET. GYNECOL. 121: 1124, 1975) is hoist with his own petard. He takes Drs. Whitehouse and Mountrose (AM. J. OBSTET. GYNECOL. 116: 880, 1973) rather severely to task for not quoting his paper on renal agenesis (Sem. Hop. Paris 43e: 926, 1967), but if *he* "had made a serious review of the literature he would probably have noticed that I have published a paper" also on that subject (J. Obstet. Gynaecol. Br. Commonw. 62: 592, 1955). It is not one that I am now particularly proud of, especially in comparison with Dr. Dellenbach's, but perhaps in the latter's 59 references another small one written in English would not have been amiss? In the vast ocean of modern medical literature, all authors, whatever their mother tongue, rather tend to navigate in glass boats—and therefore should avoid the use of brickbats!

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Reply to Dr. Green

To the Editors:

Dr. Green scores a point and that should certainly close the issue.

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Role of the nurse-midwife

To the Editors:

The nurse-midwifery staff at the Frontier Nursing Service (F.N.S.) has read the Presidential address of Dr. Russell J. Paalman to the Central Association of Obstetricians and Gynecologists (AM. J. OBSTET. GYNECOL. 122: 139, 1975) with interest but also dismay. As the first nurse-midwifery service in the United States, we are justly proud of the service we as nurse-midwives have rendered to an economically deprived area in Appalachia. At the same time, we are cognizant of our relationship to medicine and the contribution it has made to our outstanding record in maternal, infant, and family care.

There has always been a reciprocal sharing of responsibilities in the care of the pregnant woman and her entire family at the F.N.S. Indeed the work of the F.N.S. has depended on: (1) the trust and confidence our doctors and patients have had in the skills and judgments of the nurse-midwife to provide care for the normal parturient woman, and (2) the ability of the nurse-midwife to give meticulous prenatal care and recognize any deviation from normal. We would also like to point out that as one of her objectives Mrs. Mary Breckinridge sought to bring health care to the people at a cost they could afford.

Currently the F.N.S. is doing its utmost to help other areas in Kentucky where physicians are facing a crisis in providing services to pregnant women. Health manpower statistics and projection vary with every author, but the concerned voices of physicians asking for our assistance tell us that there is a real problem in this state.

Another matter of concern to all of us as health professionals is the growing demand on the part of the pregnant woman and indeed all women to play an active role in their own health care. Slowly but surely, women as consumers are demanding a different type of care and are beginning to recognize the unique role the nurse-midwife plays in normal childbirth. The growing expectations of an enlightened public demand that all health professionals realize the different potentialities that each of us possess and use them appropriately.

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Reply to Miss Fenton

To the Editors:

I am grateful that the staff of the Frontier Nursing Service read my Presidential address, given last October in New Orleans. I am sorry that you used the word "dismay" when you talk about this address. But I certainly do understand your feelings, knowing how deeply involved you are in delivering babies in Kentucky.

If you read the address carefully, you will note that I said something to the effect that "except in certain unusual places" I thought there was no future for nurse-midwifery in the United States. An "unusual" place certainly is the frontier of Kentucky.

I am very well acquainted with your work and have been for some time. For the past ten years I have been deeply involved in the mission program of the Reformed Church in America. We support the Institute at Annville, Kentucky. Some of our nurses have gone out to Annville and from there into your Frontier Nursing Service over the past 25 years. I think of two of our nurses particularly, one who is working in our mission field in Arabia and another who is working in the mission field in Ethiopia. Both of these nurses went through your program and received their obstetric training there. These two missionaries of ours do very fine jobs in their respective areas.

But the obstetric services available in Africa, Arabia, and the frontier of Kentucky are certainly different from the run-of-the-mill ones available in metropolitan areas throughout this country. These are the areas of which I spoke. Also noted in the address is the fact that there should be a very adequate number of obstetricians by the year 1980 in the United States. These well-trained men and women will be able to deliver 99 per cent of the babies born in this country. It is in these areas where the help of a nurse-midwife might be questioned.

I trust that your efforts in the area in Kentucky will continue to be blessed by our good Lord and that the services to the women in Kentucky will be appreciated by others as well.

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Depression of blast transformation of peripheral leukocytes by phytohemagglutinin in pregnant women

To the Editors:

The conclusion reached by Jha, Talwar, and Hingorani (Am. J. Obstet. Gynecol. 122: 965, 1975) concerning a depression of blast transformation of peripheral leukocytes by phytohemagglutinin (PHA) in pregnant women as well as a suppression of blast responsiveness of leukocyte cultures from nonpregnant women in pregnancy plasma may be misleading because of the techniques and methodologies employed.

The use of gravity sedimentation to separate peripheral leukocytes from red cells results in a population of lymphocytes (the cell which is PHA responsive) heavily and variably contaminated with polymorphonuclear leukocytes (PMN's). The PMN count doubles in pregnancy while the lymphocyte count remains relatively constant. Therefore, when Jha and his colleagues used a constant number of cells (but not a constant number of lymphocytes) in their assay, they would have a decreased number of PHA-responsive cells from the pregnant patients present; therefore, it would be expected that the uptake of tritiated thymidine (the method by which blast tranformation was measured) would be depressed compared to uptake in nonpregnant control subjects. However, this apparent depression may not be due to a defect of the pregnant lymphocyte but rather may be because fewer lymphocytes are present to be stimulated. This same kind of methodologic error is present in one of the studies[1] they cite as supporting their general conclusions. However, the authors of the article cited allow for this interpretation of their data by the following statement: "The similarity in shape of the two dose response curves (pregnant and nonpregnant) could be interpreted as indicating that the response of lymphocytes to PHA in pregnancy is not qualitatively different, but that there are simply fewer T cells (lymphocytes) to be stimulated."

With regard to the assumed depression by pregnancy plasma of blast tranformation of lymphocytes from nonpregnant women, the results obtained may be a function of the methodology employed rather than a real depressive effect by this plasma. Mangi and associates[2] have shown that serum from a large number of healthy control subjects when added to cultures of lymphocytes from one subject may produce a two-log scatter of the standard cell's response to mitogens and antigens. On the basis of this finding and because of a similar result in a study of cell-mediated immunity in women using oral contraceptives,[3] I do not believe that immunosuppressive properties of a given serum can be sought by stimulating normal cells in one serum and comparing the response to that obtained in another. In order to demonstrate active suppression (as opposed to nutritive or otherwise passive inability to support cell growth) of a given sera, the sera in question must demonstrate suppression when added to cells and normal pooled sera. The findings of Jha and colleagues may simply express the variability of the ability of various allogenic sera to support cell growth rather than a biologically significant immunosuppressive factor present in the sera or plasma of pregnant women.

Jha and his colleagues correctly point to the importance of the question regarding cell-mediated immunity in the pregnant state as well as to the conflicting results obtained by several investigators regarding whether or not cell-mediated immunity (CMI) as measured by lymphocyte responsiveness to PHA is depressed.[1, 4–7] The importance of the question requires that the methodologies used and the different interpretations possible because of these methodologies be clearly recognized. In addition to the problems mentioned above, further problems with interpretation arise in various studies because of the following:

1. Those methods which utilize morphologic criteria of grain count on autoradiography to assess lymphocyte blast tranformation suffer from inaccuracy due to clumping of the cells by PHA, nonspecific vacuolation,

and the counting of cells whether they are dead or alive.[1, 7]

2. Various authors use either total tritiated thymidine counts or stimulation indexes to report their results. In order for studies to be comparable, both measures should be used and reported.[4–7]

3. Studies that do not heat inactivate sera and, therefore, have complement in addition to allogenic sera as part of the system allow for the possibility of complement-mediated cell destruction to adversely influence the results obtained.[1]

In summary, the question of whether cell-mediated immunity as measured by PHA lymphocyte stimulation is depressed in pregnancy has not been answered as clearly as the paper by Jha and colleagues would imply. Until methodologies and reporting are standardized and these standardized methods are used to follow serially a population of women acting as their own controls (i.e., during prepregnant, pregnant, and postpartum periods), the answer to this important question will probably remain in doubt.

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Reply to Dr. Knox

To the Editors:

Dr. Knox has a point. The increase in the proportion of neutrophils in the leukocyte fraction would reduce marginally the percentage of lymphocytes in the fraction. However, we are not sure whether the PMN count doubles in pregnancy. Andrews and Bonsnes[1] reported from observations on 32 nonpregnant and 75 pregnant women that the neutrophils increased from 63 ± 8 to 72 ± 7 per cent, while the lymphocytes decreased from 29 ± 8 to 21 ± 6 per cent. There is thus a decrease of about 30 per cent in the proportion of lymphocytes in this fraction. This will contribute substantially but not explain fully the observed reduction in the PHA-induced incorporation of ^{3}H-thymidine by leukocytes obtained from the peripheral blood of pregnant women.

However, the data relating to the presence of factors in pregnant sera that inhibit the blast transformation of peripheral leukocytes with phytomitogens would be unaffected by the above argument because the effect of such sera was tested on a common preparation of cells. In follow-up studies,[2, 3] the sera from 13 pregnant women have been tested on the transformation of common-donor leukocytes. Also the effect of pregnancy serum from a given subject has been studied on the leukocytes from seven subjects. In all these cases inhibition varying from 64.7 to 99.5 per cent in transformation was observed. The inhibition was a function of the amount of pregnancy serum added in the over-all total of 20 per cent human AB serum in the medium. The inhibition of transformation by pregnancy serum was not due to cytotoxic factors, as the viability of these cells was not altered by inclusion of the serum in the culture medium. The sera used in all these studies were decomplemented. It may also be mentioned that the inhibition of transformation of leukocytes is relieved by extensive washings of the cells, suggesting that the transformation of these cells may be blocked by some factors present in the pregnancy serum which coat a subpopulation of the cells.

The modulation of maternal immune responses in such a manner that rejection of the conceptus is prevented is a complex phenomenon, and no doubt much remains to be further investigated.

G. P. Talwar

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New Delhi, 110016, India

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Dangers of nitrous oxide pneumoperitoneum

To the Editors:

In the "Correspondence" section (Am. J. Obstet. Gynecol. 121: 575, 1975), Dr. John M. Esposito commented on the dangers of nitrous oxide pneumoperitoneum in laparoscopy. In his letter, he quoted a personal communication with me regarding the dangers of nitrous oxide. His letter inferred that I

Table I. Pro's and con's of carbon dioxide and nitrous oxide

Pro	*Con*
Carbon dioxide	
1. Does not support combustion or explosion	1. Is not as readily available in most operating rooms
2. Is rapidly absorbed from the abdomen cavity, reducing chances of postoperative shoulder pain	2. Hypercarbia is a threat if assisted ventilation is not adequate (1-5)
Nitrous oxide	
1. Is readily available in most operating rooms	1. Supports combusion slightly more than common air
2. Is absorbed from abdominal cavity without affecting P_{CO_2}	2. Is absorbed slower than CO_2 from abdominal cavity
3. Hypercarbia is not a threat, particularly in the nonintubated patient[6, 7]	
4. Is not explosive	

was in support of his concept that nitrous oxide is not the insufflation medium of choice in laparoscopy with electrocoagulation. This letter is to correct that inference.

In the Greater Seattle area, nitrous oxide has been the sole insufflation gas in over 5,000 laparoscopies with electrocoagulation. To this date, no accidents have occurred because of the use of nitrous oxide. As a member of the Complications Committee of the American Association of Gynecologic Laparoscopists, we have, to this date, heard of no accidents with the use of nitrous oxide. In 1974, a survey of over 200,000 laparoscopies was conducted by the American Association of Gynecologic Laparoscopists. Approximately 20,000 laparoscopy procedures with the use of nitrous oxide were reviewed, and no accidents had occurred.

In my own institution, an individual performing his first laparoscopy sterilization with the use of an operating laparoscope and a high-voltage, spark-gap coagulation unit created an arc between the tip of the electrode and the end of the laparoscope. This operator error went unnoticed for several moments until the end of the laparoscope heated to the point that the sealant at the end of the laparoscope burst into a small flame. There was no injury to the patient from this flame, and there was no explosive problem. Had this individual been knowledgeable about operative laparoscopy and not used a high-voltage spark gap coagulation unit, it would have been impossible for this event to occur.

In 1973, I was asked by the American Association of Gynecologic Laparoscopists to review the literature in reference to the problems of carbon dioxide versus nitrous oxide. A brief synopsis of the findings from the literature is presented in Table I.

The severe cardiac arrythmias from carbon dioxide insufflation are well known. In Seattle, one cardiac arrest has occurred from hypercarbia secondary to carbon dioxide and underventilation. I have no quarrel with those who use carbon dioxide, provided they understand the risks of hypercarbia and communicate this to the anesthesiologist. The hypothetical danger, as outlined by Dr. Esposito, continues to be only hypothetical in a large series of cases reviewed.

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Reply to Dr. Soderstrom

To the Editors:

I did not wish to infer in my original letter that Dr. Soderstrom supported the concept that carbon dioxide is the insufflation medium of choice when electrocoagulation is being performed. I only wished to bring to the attention of the practicing laparoscopist a case which Dr. Soderstrom had reported to me in a personal communication. This case is the same one to which he refers in his recent letter.

It is still my opinion that if nitrous oxide had been used the flame that Dr. Soderstrom mentioned would not have taken place because carbon dioxide does not support combustion. When we speak of supporting combustion, we do not mean there will be an explosion; we only mean that if matter can burn it will burn in an atmosphere saturated with nitrous oxide and not in one saturated with carbon dioxide.

Dr. Soderstrom states that the complication occurred because an inexperienced laparoscopist performed the

operation. This may be true, and my continuing correspondence on the subject has been so that the inexperienced laparoscopist would be made aware of the danger of nitrous oxide.

I do not wish to continually be repetitive in my remarks, but once again I must state that in the past a simple theoretical fact was overlooked. Because of this initial failure to recognize the potential hazard of spark-gap–generated currents, intestinal burns occurred. The hazard did not reveal itself immediately, and numerous case reports were presented without incidence.

Although severe cardiac arrythmias have been reported from carbon dioxide insufflation, one cannot be sure whether the hypercarbia developed because of the CO_2 absorbed from the peritoneal cavity or because of inadequate ventilation.

In fact, in the paper entitled "Nitrous oxide and carbon dioxide for laparoscopy in a randomized manner. The effect on gas exchange and respiration," by Stephens, R., and Thompson, B., presented at the 1974 Symposium of Gynecologic Endoscopy in Anaheim, California, no difference was shown between the two gases in the production of hypercarbia if the respiratory exchange was adequate.

I think it is our duty to make the problems known so that physicians may evaluate both methods and make their decision. Once the decision is made, they may then intelligently use the method selected. However, I do feel that hypercarbia is something that can be avoided and that with proper monitoring steps can be taken early to remedy the situation, if and when it occurs.

Interperitoneal combustion, such as occurred in the case described by Dr. Soderstrom, is a complication which we have no means of monitoring. Possibly, the theoretical problem of flash fire in the peritoneal cavity will never occur. However, I think it must be taken into consideration by all who wish to perform electrosurgical procedures under laparoscopic supervision with nitrous oxide used to establish pneumoperitoneum.

In Dr. Soderstrom's brief synopsis of the pro's and con's for carbon dioxide and nitrous oxide, the following should be taken into consideration: (1) Hypercarbia can be a threat even with nitrous oxide pneumoperitoneum if ventilation and respiratory exchange are not adequate: (2) it does not matter if nitrous oxide supports combustion only slightly more than common air (in fact, it does support combustion and carbon dioxide does not), (3) It is not a pro for nitrous oxide that it does not explode because neither does carbon dioxide.

Dr. Soderstrom presented a case of cardiac arrest second to hypercarbia. I think the key terminology in the etiology of the death is "underventilation" and not "carbon dioxide pneumoperitoneum."

John M. Esposito, M.D.
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Brooklyn, New York 11209

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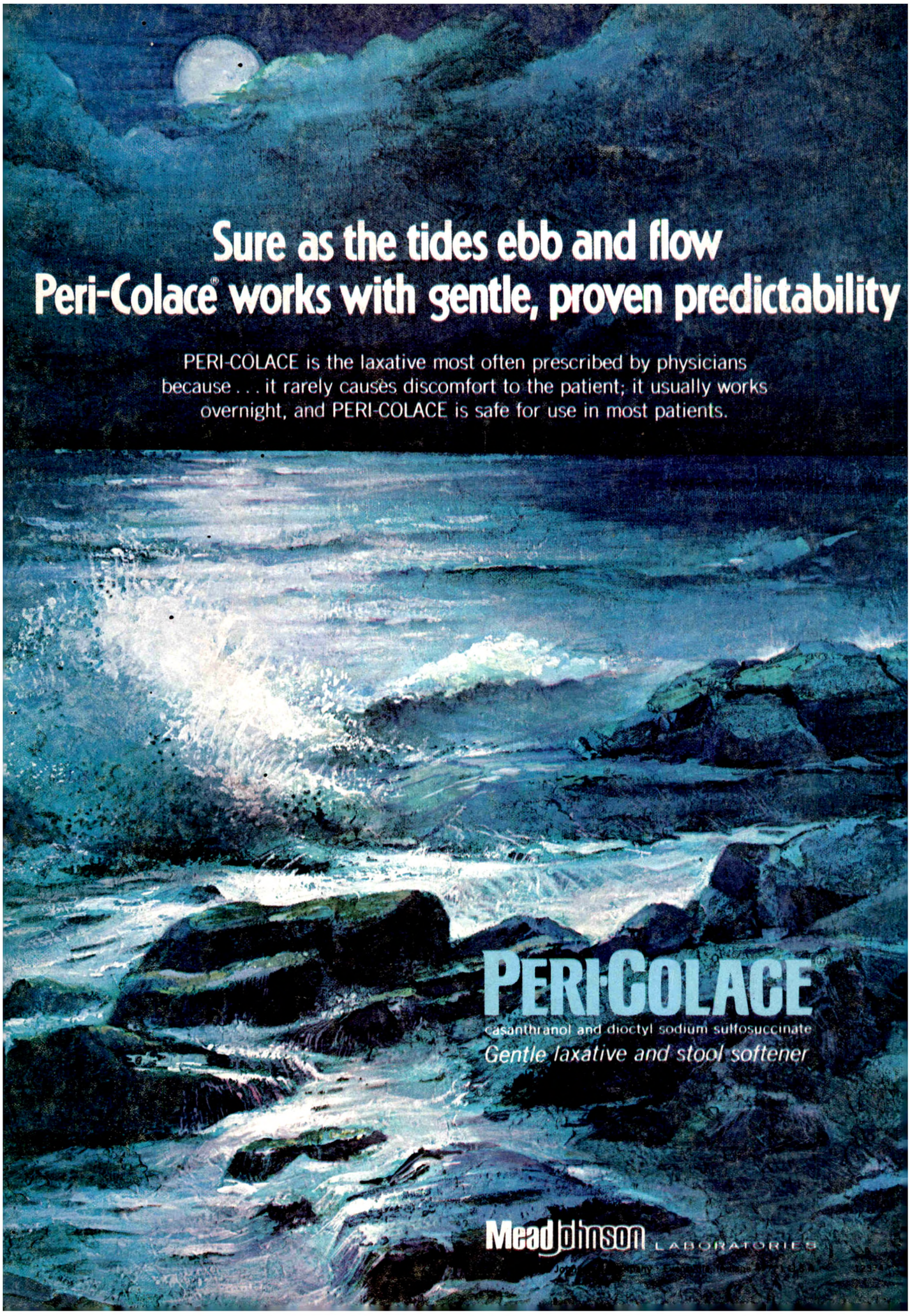
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Long term administration of both natural and synthetic estrogens in subprimate animal species in multiples of the human dose increases the frequency for some animal carcinomas. These data cannot be transposed directly to man. The possible carcinogenicity due to the estrogens can neither be affirmed nor refuted at this time. Close clinical surveillance of all women taking oral contraceptives must be continued.

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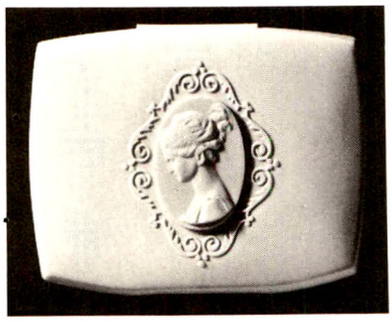
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1. Thrombophlebitis, thromboembolic disorders, cerebral apoplexy, or a past history of these conditions.
2. Markedly impaired liver function.
3. Known or suspected carcinoma of the breast.
4. Known or suspected estrogen dependent neoplasia.
5. Undiagnosed abnormal genital bleeding.
6. Known or suspected pregnancy.

Warnings

1. The physician should be alert to the earliest manifestations of thrombotic disorders (thrombophlebitis, cerebrovascular disorders, pulmonary embolism, and retinal thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. Retrospective studies of morbidity and mortality in Great Britain and studies of morbidity in the United States have shown a statistically significant association between thrombophlebitis, pulmonary embolism, and cerebral thrombosis and embolism and use of oral contraceptives. There have been three principal studies in Britain[1,2,3] leading to this conclusion, and one[4] in this country. The estimate of the relative risk of thromboembolism in the study by Vessey and Doll[3] was about sevenfold, while Sartwell and associates in the United States found a relative risk of 4.4, meaning that the users are several times as likely to undergo thromboembolic disease without evident cause as non-users. The American study also indicated that the risk did not persist after discontinuation of administration, and that it was not enhanced by long continued administration. The American study was not designed to evaluate a difference between products. However, the study suggested that there might be an increased risk of thromboembolic disease in users of sequential products. This risk cannot be quantitated, and further studies to confirm this finding are desirable.
2. Discontinue medication pending examination if there is sudden partial or complete loss of vision, or if there is a sudden onset of proptosis, diplopia or migraine. If examination reveals papilledema or retinal vascular lesions, medication should be withdrawn.
3. Since the safety of NORINYL 1+50 and NORINYL 1+80 in pregnancy has not been demonstrated, it is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule the possibility of pregnancy should be considered at the time of the first missed period.
4. A small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The long range effect to the nursing infant cannot be determined at this time.
5. Hepatic cell adenomas have been reported in women on oral contraceptives. The adenoma may present as an abdominal mass or with the signs and symptoms of an acute abdomen. A bleeding hepatic cell adenoma should be considered if the patient has abdominal pain or evidence of intra-abdominal bleeding.



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Precautions

1. The pretreatment and periodic physical examinations should include special reference to liver, breasts and pelvic organs, including Papanicolaou smear since estrogens have been known to produce tumors, some of them malignant, in five species of subprimate animals.
2. Endocrine and possibly liver function tests may be affected by treatment with NORINYL 1+50 and NORINYL 1+80. Therefore, if such tests are abnormal in a patient taking NORINYL 1+50 and NORINYL 1+80, it is recommended that they be repeated after the drug has been withdrawn for 2 months.
3. Under the influence of estrogen-progestogen preparations, pre-existing uterine fibromyomata may increase in size.
4. Because these agents may cause some degree of fluid retention, conditions which might be influenced by this factor, such as epilepsy, migraine, asthma, cardiac or renal dysfunction, require careful observation.
5. In breakthrough bleeding, and in all cases of irregular bleeding per vaginam, nonfunctional causes should be borne in mind. In undiagnosed bleeding per vaginam, adequate diagnostic measures are indicated.
6. Patients with a history of psychic depression should be carefully observed and the drug discontinued if the depression recurs to a serious degree.
7. Any possible influence of prolonged NORINYL 1+50 and NORINYL 1+80 therapy on pituitary, ovarian, adrenal, hepatic or uterine function awaits further study.
8. A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. The mechanism of this decrease is obscure. For this reason, diabetic patients should be carefully observed while receiving NORINYL 1+50 and NORINYL 1+80 therapy.
9. The age of the patient constitutes no absolute limiting factor, although treatment with NORINYL 1+50 and NORINYL 1+80 may mask the onset of the climacteric.
10. The pathologist should be advised of NORINYL 1+50 and NORINYL 1+80 therapy when relevant specimens are submitted.
11. Susceptible women may experience an increase in blood pressure following administration of contraceptive steroids.

Adverse Reactions Observed in Patients Receiving Oral Contraceptives

A statistically significant association has been demonstrated between use of oral contraceptives and the following serious adverse reactions: thrombophlebitis, pulmonary embolism, cerebral thrombosis.

Although available evidence is suggestive of an association, such a relationship has been neither confirmed nor refuted for the following serious adverse reactions: neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions are known to occur in patients receiving oral contraceptives: nausea, vomiting, gastrointestinal symptoms (such as abdominal cramps and bloating), breakthrough bleeding, spotting, change in menstrual flow, amenorrhea during and after treatment, edema, chloasma or melasma, breast changes: tenderness, enlargement and secretion, change in weight (increase or decrease), changes in cervical erosion and cervical secretions, suppression of lactation when given immediately post-partum, cholestatic jaundice, migraine, rash (allergic), rise in blood pressure in susceptible individuals, mental depression.

Although the following adverse reactions have been reported in users of oral contraceptives, an association has been neither confirmed nor refuted: anovulation post treatment, premenstrual-like syndrome, changes in libido, changes in appetite, cystitis-like syndrome, hirsutism, loss of scalp hair, erythema multiforme, headache, nervousness, dizziness, fatigue, backache, erythema nodosum, hemorrhagic eruption, itching.

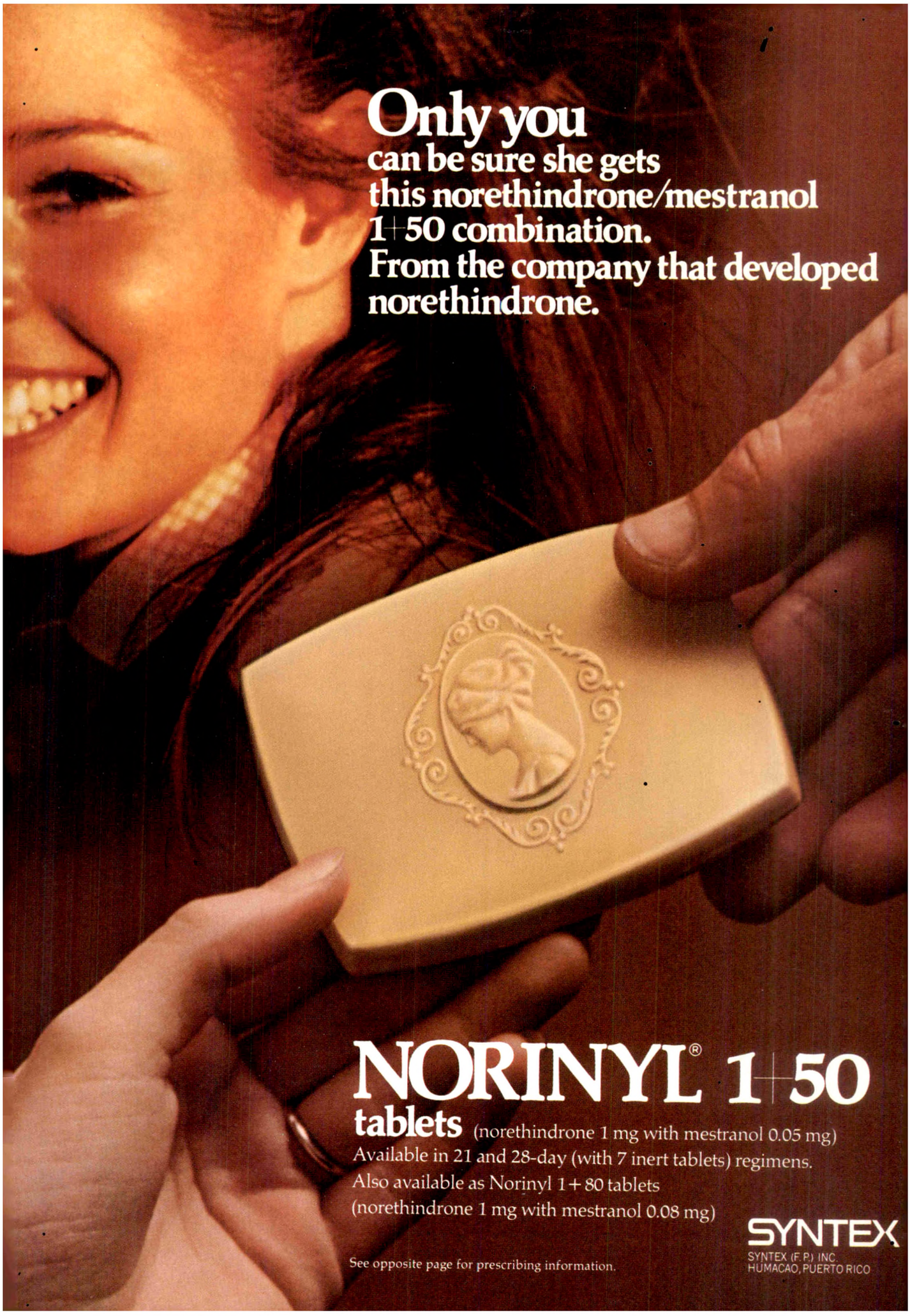
The following laboratory results may be altered by the use of oral contraceptives: hepatic function: increased sulfobromophthalein retention and other tests, coagulation tests: increase in prothrombin factors VII, VIII, IX, and X, thyroid function: increase in PBI, and butanol extractable protein bound iodine and decrease in T_3 uptake values, metyrapone test, pregnanediol determination.

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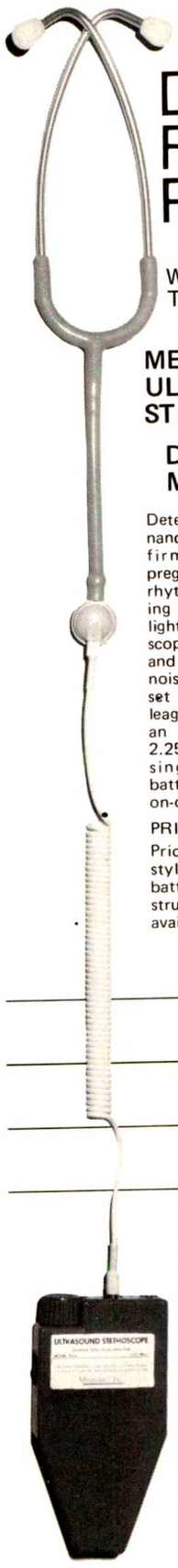
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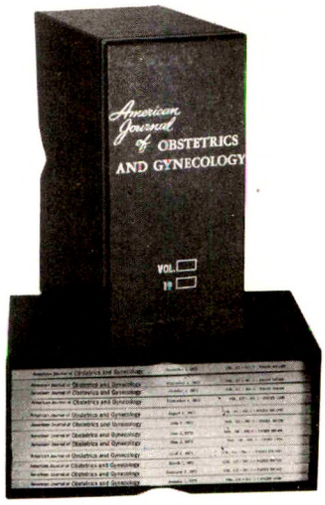
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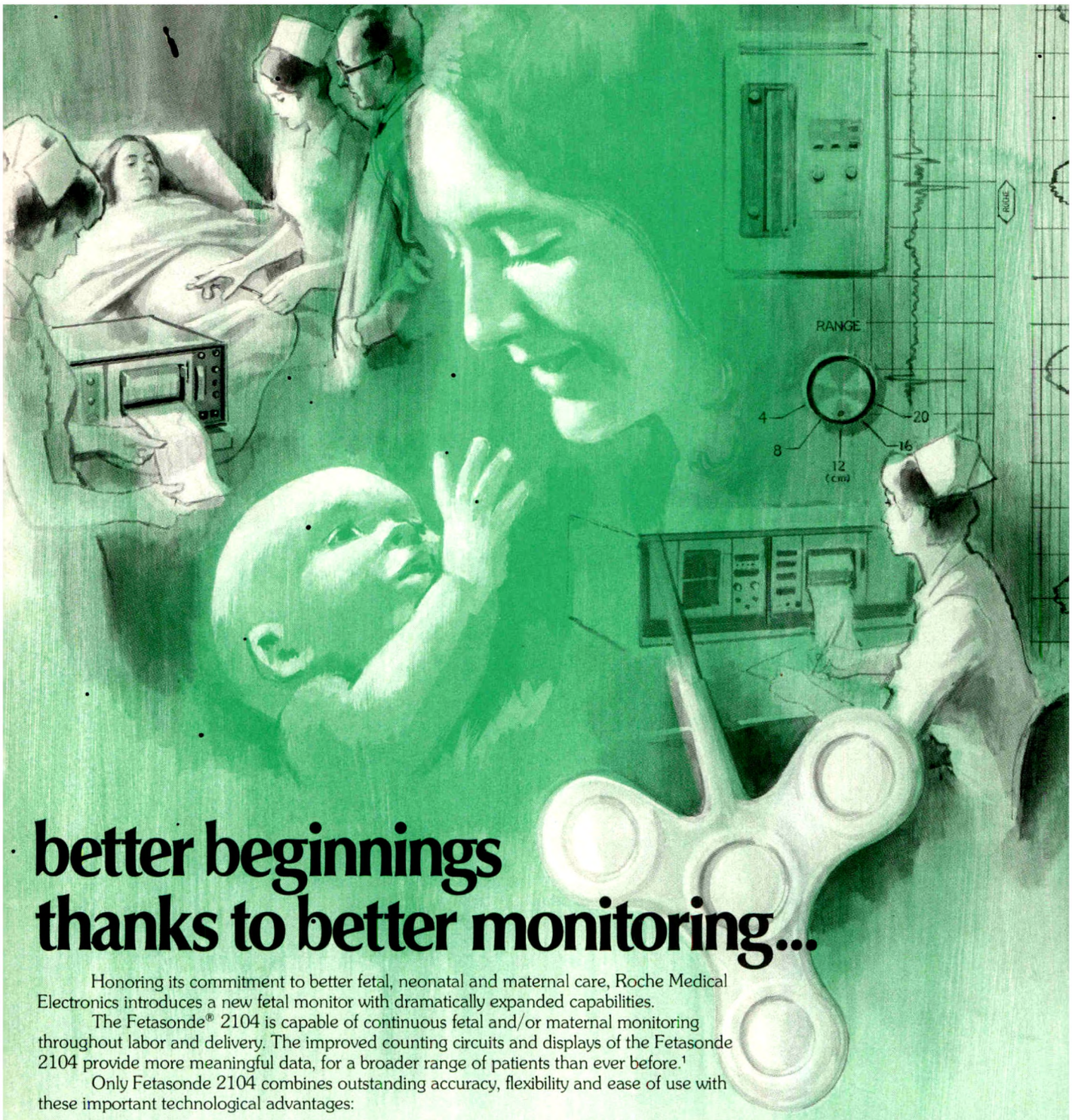
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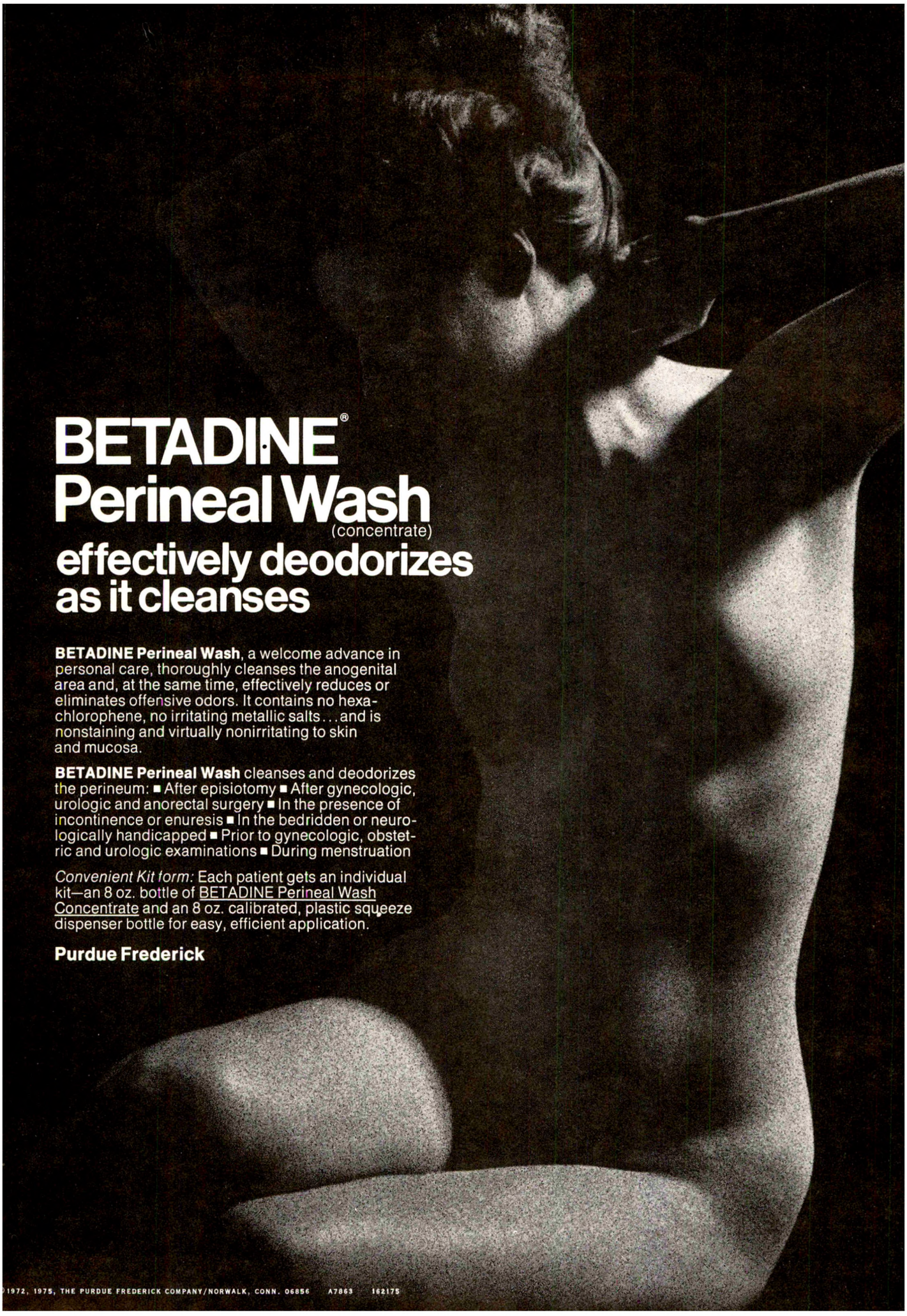
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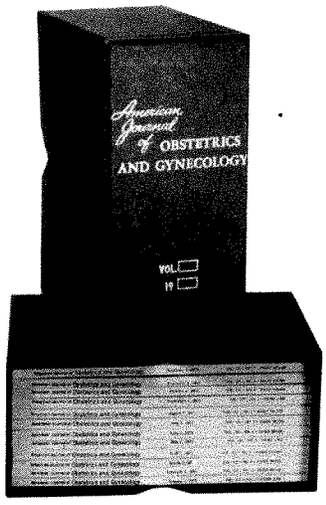
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Breakthrough Bleeding	9.0%	3.5%	2.7%	2.6%	3.9%

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*Serious as well as minor adverse reactions have been reported following the use of all oral contraceptives. The physician should remain alert to their earliest manifestations and in the event of serious adverse reactions should avoid any oral contraceptives. *Special note, contraindications, warnings, precautions and adverse reactions* in the accompanying IN BRIEF should be carefully considered.

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Low dose oral contraceptive.

IN BRIEF:

SPECIAL NOTE: Oral contraceptives have been marketed in the United States since 1960. Reported pregnancy rates vary from product to product. The effectiveness of the sequential products appears to be somewhat lower than that of the combination products. Both types provide almost completely effective contraception.

An increased risk of thrombotic, thromboembolic and cerebral-vascular disease associated with the use of hormonal contraceptives has been shown in studies conducted in both Great Britain and the United States. Other risks, such as those of elevated blood pressure and reduced tolerance to carbohydrates, have been reported, and appropriate tests should be conducted to monitor these during oral-contraceptive therapy. Liver disease has also been reported, and the physician should be alert to its earliest manifestations.

Long-term administration of both natural and synthetic estrogens in subprimate animal species in multiples of the human dose increases the frequency for some animal carcinomas. These data cannot be transposed directly to man. The possible carcinogenicity due to the estrogens can neither be affirmed nor refuted at this time. Close clinical surveillance of all women taking oral contraceptives must be continued.

INDICATIONS: For oral contraception.

CONTRAINDICATIONS: 1. Thrombophlebitis, thromboembolic disorders, cerebral-vascular disease, or a past history of these conditions; 2. Markedly impaired liver function; 3. Known or suspected carcinoma of the breast; 4. Known or suspected estrogen-dependent neoplasia; 5. Undiagnosed abnormal genital bleeding; 6. Known or suspected pregnancy.

WARNINGS: 1. The physician should be alert to the earliest manifestations of thrombotic and thromboembolic disorders, thrombophlebitis, cerebrovascular disorders including hemorrhage, pulmonary embolism and retinal thrombosis. Should any of these occur or be suspected, the drug should be discontinued immediately.

Retrospective studies of morbidity and mortality in Great Britain and studies of morbidity in the United States have shown a statistically significant association between thrombophlebitis, pulmonary embolism and cerebral-vascular disease, occlusive or hemorrhagic, and the use of oral contraceptives. There have been three principal studies in Great Britain[1-3] leading to these conclusions and three[4-7] in this country. The estimate of the relative risk of thromboembolism in the study by Vessey and Doll[3] was about sevenfold, while the United States studies found relative risks of 4.4 to 11, meaning that the users are several times as likely to undergo thromboembolic disease without evident cause as non-users.

In May, 1974, the Royal College of General Practitioners in England[8] issued an interim report of its continuing large-scale prospective study comparing a user group to a non-user group. This study in its interim analysis states: "A statistically significant higher rate of reporting of cerebrovascular accidents in Takers is evident, but the numbers are too small to justify an estimation of the degree of risk." The study also reported a higher incidence of superficial and deep vein thrombosis in users as compared to non-users.

The Sartwell study[4] indicated that the risk did not persist after discontinuation of administration. Both the Sartwell and the Royal College study indicated that the degree of risk was not associated with duration of treatment.

In a collaborative American study[5,6] of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the relative risk of thrombotic stroke was 4.1 to 9.5 times greater in users than in non-users. A comparable estimate for hemorrhagic stroke was 2.0.

None of the American studies was designed to evaluate a difference between products. However, the Sartwell study[4] suggested that there might be an increased risk of thromboembolic disease in users of sequential products.

Other retrospective studies[9,10] have reported an increased risk of post-surgery thromboembolic complications in oral-contraceptive users. It has been recommended that therapy be discontinued at least one month prior to elective surgery.

2. Discontinue oral-contraceptive medication if there is: gradual or sudden, partial or complete loss of vision; proptosis or diplopia; onset or aggravation of migraine or development of headache of a new pattern which is recurrent, persistent or severe; papilledema; or any evidence of retinal-vascular lesions. 3. Fetal abnormalities have been reported to occur in the offspring of women who have taken progestogens and/or estrogens during pregnancy[11,12] The safety of LO/OVRAL® in pregnancy has not been demonstrated. Pregnancy should be ruled out before initiating or continuing the contraceptive regimen. Pregnancy should always be considered if withdrawal bleeding does not occur. 4. A small fraction of the hormonal agent in oral contraceptives has been identified in the milk of mothers receiving these drugs. The long-range effect to the nursing infant cannot be determined at this time. 5. Hepatic lesions (adenomas, hepatomas, hamartomas, regenerating nodules, etc.), occasionally fatal, have been reported in women on oral contraceptives. Such lesions may present as an abdominal mass or with the signs and symptoms of an acute abdomen. These lesions should be considered if the patient has abdominal pain or evidence of intra-abdominal bleeding. Such lesions have been reported in short-term as well as long-term users of oral contraceptives.

PRECAUTIONS: 1. A thorough history and physical examination should be performed before prescribing oral contraceptives and periodically during their administration and should include special reference to breasts and pelvic organs including Papanicolaou smears, since estrogens have been known to produce tumors, some of them malignant, in five species of subprimate animals. 2. Endocrine and possibly liver-function tests may be affected by treatment with LO/OVRAL® Therefore, if such tests are abnormal in a patient taking LO/OVRAL® it is recommended that they be repeated after the drug has been withdrawn for two months. 3. Under the influence of estrogen-progestogen preparations, pre-existing uterine fibromyomata may increase in size. 4. Because these agents may cause some degree of fluid retention, conditions which might be influenced by this factor, such as epilepsy, migraine, asthma, cardiac or renal dysfunction, require careful observation. 5. In breakthrough bleeding, and in all cases of irregular bleeding per vaginam, nonfunctional causes should be borne in mind. In undiagnosed bleeding per vaginam, adequate diagnostic measures are indicated. 6. Patients with a history of psychic depression should be carefully observed and the drug discontinued if the depression recurs to a serious degree. 7. Any possible influence of prolonged LO/OVRAL® therapy on pituitary, ovarian, adrenal, hepatic or uterine function awaits further study. 8. A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. The mechanism of this decrease is obscure. For this reason, diabetic patients should be carefully observed while receiving LO/OVRAL® therapy. 9. The age of the patient constitutes no absolute limiting factor, although treatment with LO/OVRAL® may mask the onset of the climacteric. 10. The pathologist should be advised of LO/OVRAL® therapy when relevant specimens are submitted. 11. Susceptible women may experience an increase in blood pressure following administration of contraceptive steroids. 12. Cholestatic jaundice has been reported in users of oral contraceptives. If this occurs, LO/OVRAL® should be discontinued. This condition is more likely to occur in patients who have experienced cholestatic jaundice of pregnancy. Patients with a history of cholestatic jaundice of pregnancy should be carefully observed during LO/OVRAL® therapy.

ADVERSE REACTIONS OBSERVED IN PATIENTS RECEIVING ORAL CONTRACEPTIVES: A statistically significant association has been demonstrated between use of oral contraceptives and the following serious adverse reactions: thrombophlebitis, pulmonary embolism, cerebral thrombosis and hemorrhage, gall bladder disease.

Although available evidence is suggestive of an association, such a relationship has been neither confirmed nor refuted for the following serious adverse reactions: neuro-ocular lesions, e.g., retinal thrombosis, optic neuritis and hepatic lesions with or without intra-abdominal bleeding.

The following adverse reactions are known to occur in patients receiving oral contraceptives: nausea, vomiting, gastrointestinal symptoms (such as abdominal cramps and bloating), breakthrough bleeding, spotting, change in menstrual flow, amenorrhea during and after treatment, edema, chloasma or melasma, which may persist, breast changes: tenderness, enlargement and secretion, change in weight (increase or decrease), changes in cervical erosion and cervical secretions, suppression of lactation when given immediately post-partum, cholestatic jaundice, migraine, rash (allergic), rise in blood pressure in susceptible individuals, mental depression.

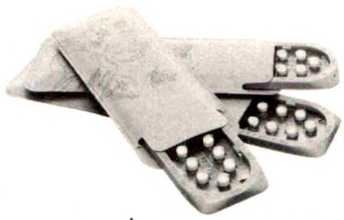
Although the following adverse reactions have been reported in users of oral contraceptives, an association has been neither confirmed nor refuted: anovulation post treatment, which tends to occur more frequently in women with a history of menstrual disorders, premenstrual-like syndrome, changes in libido, changes in appetite, cystitis-like syndrome, headache, intolerance to contact lenses, nervousness, dizziness, fatigue, backache, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, itching and vaginitis.

The following laboratory results may be altered by the use of oral contraceptives: hepatic function; increased sulfobromophthalein retention and other tests; coagulation tests; increase in prothrombin, Factors VII, VIII, IX and X; decrease in antithrombin III; increase in platelet aggregability; thyroid function: increase in PBI, and butanol extractable protein-bound iodine and decrease in T^3 uptake values; metyrapone test; and pregnanediol determination.

NOTE: For more specific details on LO/OVRAL, refer to package insert.

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OBSTETRICS

Placental ratio in white and black women: Relation to smoking and anemia

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In a study of 7,000 pregnancies it was found that, particularly in black women, hemoglobin levels were lower in smokers than in nonsmokers. This is the reverse of previously reported findings in nonpregnant persons. At delivery, the mean ratio of placental weight to birth weight (placental ratio) was higher in the infants of smokers than of nonsmokers. This observation is discussed in the light of reported elevated placental ratios at high altitudes or after severe anemia of pregnancy. The relationships are complex, and are not uniform with respect to ethnic group.

THE WELL-KNOWN association between maternal smoking and reduced birth weight does not appear to extend to a diminished placental weight. Reports have indicated that the placental ratio (ratio of placental weight to birth weight) is higher in pregnancies with smoking than in those without.[1, 2] A high placental ratio has also been observed in births at high altitudes[3] and has recently been found in women with severe anemia of pregnancy.[4] To investigate the similarities and dissimilarities between the phenomena, we have analyzed hemoglobin levels during pregnancy and placental ratios in more than 7,000 births.

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†*Deceased.*

Subjects and methods

The subjects were members of the Kaiser Foundation Health Plan in Oakland, California, whose subscribers constitute a predominantly employed urban population representing a broad range of economic, social, and educational characteristics deficient only in the very affluent and the very indigent groups. In the years 1959-1967, all women planning to deliver at the Oakland Kaiser Foundation Hospital were enrolled in the Child Health and Development Studies.[5] At an interview early in pregnancy, information was obtained

Table I. Means of birth weight, placental weight, and placental ratio by race and sex

	White		*Black*	
	Boys N = 2,790	*Girls N = 2,726*	*Boys N = 1,088*	*Girls N = 1,036*
Birth weight (Gm.)	3,495.4	3,354.0	3,333.5	3,216.0
Placental weight (Gm.)	473.1	462.1	458.5	453.5
Placental ratio × 100	13.56	13.79	13.78	14.13

Table II. Means of birth weight, placental weight, and placental ratio by race and smoking habits during pregnancy

	White			*Black*		
	Nonsmokers N = 3,459	*Light smokers N = 1,060*	*Heavy smokers N = 997*	*Nonsmokers N = 1,439*	*Light smokers N = 504*	*Heavy smokers N = 181*
Birth weight (Gm.), S.D. = 460	3,498.4	3,334.4	3,269.3	3,333.7	3,158.0	3,148.7
Placental weight (Gm.), S.D. = 90	467.6	463.2	472.3	455.2	455.1	465.9
Placental ratio × 100, S.D. = 2.2	13.37	13.90	14.48	13.65	14.45	14.93

Table III. Correlations of minimum adjusted hemoglobin level with placental weight, birth weight, and placental ratio

	White		*Black*	
	Nonsmokers	*Smokers*	*Nonsmokers*	*Smokers*
Placental weight	−0.060	−0.052	−0.050	−0.101
Birth weight	−0.050	−0.032	−0.020	−0.007
Placental ratio	−0.033	−0.036	−0.050	−0.109

about numerous factors including smoking habits. Hemoglobin was measured routinely in the medical center's prenatal clinic. The placentas were weighed by specially trained personnel after the cord and attached membranes had been trimmed off according to Benirschke's[6] protocol.

The present study was confined to women who delivered single, live infants without severe anomaly after 37 to 43 weeks' gestation, and for whom at least one hemoglobin value during gestation was reported.

Results

Birth weight, placental weight, and placental ratio, by race and sex. The widely recognized effect of race and sex on birth weight is confirmed in Table I, white infants being heavier than blacks, boys heavier than girls. Placental weight shows the same relations, but in lesser degree, with the result that the placental ratio is seen to be slightly higher for female and for black infants than for males and whites.

Placental ratio by length of gestation and smoking categories. As shown in Fig. 1, the placental ratio declined slowly between 37 and 40 weeks' gestation and remained essentially constant thereafter. The placental ratio in heavy smokers (>20 cigarettes daily) was consistently higher than in nonsmokers; this difference was distinct for each week of gestation (37 through 43). In light smokers (<20 cigarettes daily), placental ratios occupied an intermediate position between those of heavy smokers and nonsmokers. The arithmetical explanation for elevated placental ratio in smokers is evident in Table II: the decrease in birth weight that was associated with smoking was not accompanied by a similar decrease in placental weight. Armitage and associates[7] found that as birth weight increases, the placental ratio decreases. A similar pattern was present in our data, but this was entirely accounted for by the effects of race, sex, length of gestation, and smoking habits.

Hemoglobin level during pregnancy. The means of

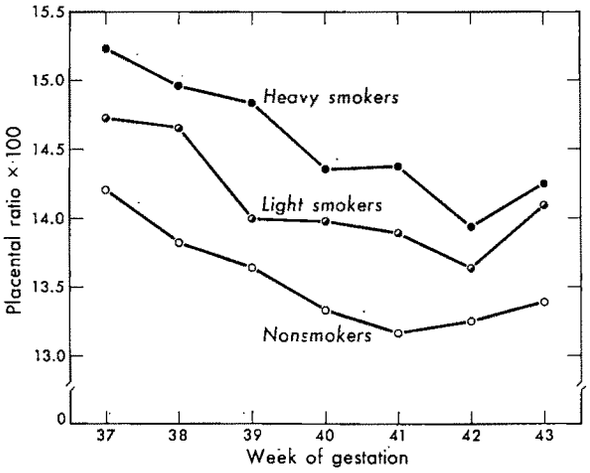


Fig. 1. Placental ratio by length of gestation and smoking category.

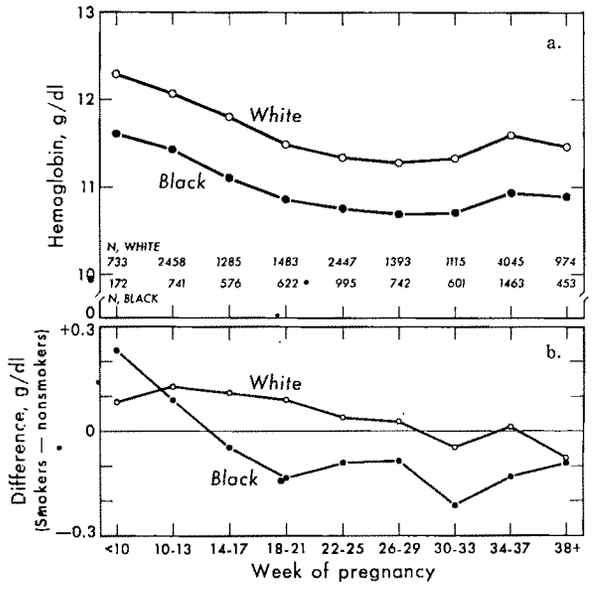


Fig. 2. Mean hemoglobin levels, and difference between mean hemoglobin levels of smokers and nonsmokers, by week of pregnancy and race.

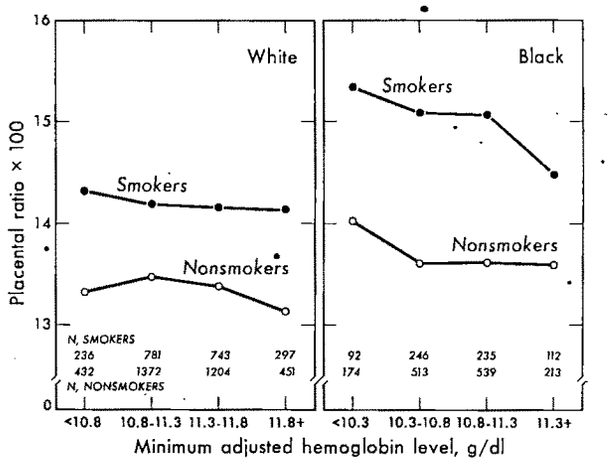


Fig. 3. Placental ratio by minimum adjusted hemoglobin level, race, and smoking category.

all hemoglobin measurements by week of gestation are shown in Fig. 2. In both races, hemoglobin declined sharply in the first trimester and rose moderately in the third. Throughout gestation, hemoglobin levels were lower in blacks than in whites.

Only minor differences in hemoglobin level between smokers and nonsmokers were found. In both races, however, there did appear to be systematic change with length of gestation (Fig. 2): in early pregnancy mean hemoglobin levels were slightly higher among smokers than among nonsmokers; this difference later disappeared among whites and was actually reversed among blacks.

Few instances of severe anemia were found in this middle-class population; there were only 254 readings below 9 Gm. per deciliter and 22 below 8 Gm. per deciliter, among 19,845 determinations. Ninety-three per cent of the readings were in the normal range of 10 Gm. per deciliter or above.

Because the hemoglobin level normally falls as pregnancy progresses, a reading that is low in absolute value implies more severe anemia if seen during early than during later pregnancy (Fig. 2). To take this time difference into account, each hemoglobin reading was adjusted, by quadratic regression, to the curves of Fig. 2; for each woman the minimum adjusted reading was taken as the measure of "degree of anemia" or relative hemoglobin level.

Relation of smoking and anemia to placental ratio. Table III shows the correlations of minimum adjusted hemoglobin level, as described above, with placental weight, birth weight, and placental ratio. In each race-smoking category there is a small but significant negative correlation between placental weight and hemoglobin level, indicating that placental weight tends to increase with increasing degree of "anemia." Surprisingly, the correlations with birth weight are also negative, although here only for white nonsmokers is the correlation significantly different from zero. The correlations with placental ratio are also very small, with the exception of black smokers, for whom the correlation (−0.11) reflects the relatively strong negative association of hemoglobin level with placental

weight coupled with the negligible association with birth weight. The difference in placental ratio between smokers and nonsmokers (Fig. 3) does not appear to be dependent upon hemoglobin level.

Comment

This study of California white and black women indicates that the placenta is relatively heavy in association with a female fetus, in black women, and in smokers: among these three factors, smoking is associated with the highest placental ratio. Investigators in Ireland[1] and in New Zealand[2] have observed a similar relation between smoking and high placental ratio. Wilson[2] suggested that smoking causes chronic fetal hypoxia, resulting in small fetal size, and that the relative placental hypertrophy is compensatory. This hypothesis is supported by the increased placental ratio observed where gestation is at high altitude or the mother is severely anemic.

Our data neither totally confirm nor totally refute this hypothesis. The present finding that hemoglobin levels were not elevated among the smokers in this study can be interpreted in support of this hypothesis. In nonpregnant subjects, hemoglobin levels have been reported to be 0.3 to 0.5 Gm. per deciliter higher in smokers than in nonsmokers[8, 9]; this is apparently due to increased mean corpuscular volume, rather than to a higher red blood cell count. At no stage in pregnancy were differences of this magnitude found in the present series (Fig. 2); on the contrary, it appeared that, among black women at least, the mean hemoglobin level of smokers actually fell below that of nonsmokers as pregnancy progressed. Similarly, Rush and Kass,[10] who recorded hematocrit levels at around the twentieth week of gestation, observed slightly lower levels in smokers than in nonsmokers. It appears that during pregnancy there may be a failure of the normal compensation for the effect of smoking, with resulting functional anemia.

But the view that women who smoke are functionally anemic because a portion of their hemoglobin is bound by carbon monoxide cannot be entirely reconciled with our data. The high placental ratios observed in severe pregnancy anemia and at high altitude have been the result not only of reduced birth weight but also of increased placental weight. In neither the study of Mulcahy and associates[1] nor of Wilson[2] was smoking found to be associated with an absolute increase in placental weight; similarly, little evidence of placental hypertrophy was found in the present study (Table II); the increase in placental ratio appears to be due almost entirely to reduced birth weight.

Further, if the concept of functional anemia were correct, the elevation in placental ratio associated with smoking should be greatest in those women with the lowest hemoglobin levels. We observed increased placental ratios in smokers irrespective of hemoglobin level (Fig. 3), and while this may be due to the fact that few cases of even borderline anemia existed in this population, it also suggests that the effects of anemia and smoking upon the placental ratio are not identical.

An exception to the lack of correlation between placental ratio and anemia was found in the black women who smoked: in them, anemia was associated with a higher placental ratio. Since the hemoglobin level at all stages of pregnancy is lower in black women than in whites, blacks may be more vulnerable to anemia if they smoke.

Although it is known that infants of white smoking mothers have diminished birth weight, a corresponding increase in mortality rate has not been consistently found.[8] In black women, however, there is evidence of higher fetal and perinatal mortality rates among smokers than among nonsmokers.[10, 11] Lubs[11] has suggested that heightened risk in black women might be explained by heterozygosity for sickle cell anemia, or for glucose-6-phosphate dehydrogenase deficiency. Confirmation of this hypothesis requires further study, since many environmental differences are also involved; but the fact that black women who smoke during pregnancy are in a special category is confirmed by the hemoglobin and placental ratio findings reported here.

In any case, due caution should be observed in drawing conclusions from a purely observational study such as the present one. Women who smoke are a self-selected group, who differ in many respects from those who do not. Hypoxia may well be involved in the observations reported here, but the physiologic mechanisms are undoubtedly complex and cannot be completely elucidated from an observational study.

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Teratogenic effects of ethyl alcohol administered to pregnant mice

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Concern has recently been renewed about possible detrimental effects of maternal alcoholism on human prenatal development. In light of current interest in maternal alcoholism, data are presented on the effects of ethyl alcohol administration to pregnant mice. Alcohol treatment consisted of intraperitoneal injection of 0.030 ml. per gram of body weight of a 25 per cent (v/v) solution of 95 per cent ethyl alcohol in saline. Following alcohol treatment on both of gestation days 8 and 9 or days 10 and 11 the frequency of fetal death was substantially elevated compared to saline-treated controls. Alcohol treatment on one of gestation days 7, 8, 9, 10, 11, or 12 resulted in significantly increased fetal death on days 9, 10, 11, and 12; there was a marginally significant increase in fetal death following alcohol treatment on day 8. The incidence of congenital anomalies was significantly increased following alcohol treatment only on gestation days 8, 9, or 10. The most frequently observed abnormalities were coloboma of the iris and ectrodactyly affecting the forepaws.

ETHYL ALCOHOL is probably the most widely abused drug in North America.[1] A number of recent clinical reports have renewed concern about possible teratogenic effects of alcohol in human beings.[2–7] These reports have described a spectrum of congenital anomalies, called the fetal alcohol syndrome, which can include deficient intrauterine and postnatal growth, ocular, joint, and cardiac anomalies, and possible psychomotor retardation. The retrospective surveys briefly reported by Ulleland[8] and Jones and associates[7] provide evidence that many of the children of severely alcoholic mothers have characteristics of the "fetal alcohol syndrome." Although these reports are suggestive of a possible causal relationship between maternal alcoholism and offspring congenital defects, no adequate epidemiologic studies have clearly demonstrated such a relationship. Green[9] has recently reviewed much of the older literature on maternal alcoholism and has highlighted the lack of information concerning the effects of ethanol on mammalian development.

In light of the current concern and uncertainty about the effects of maternal alcohol use on the unborn infant, the following report of alcohol-induced teratogenesis in the mouse is of interest.

Materials and methods

Animals. All mice used in these studies were the progeny (F_1) of a cross of the C57BL/6J (female) and DBA/2J (male) inbred strains. Females mated between 130 and 147 days of age (see below) were born and raised in the laboratory; all other females used were purchased from the Jackson Laboratory, Bar Harbor, Maine (strain B6D2F_1/J). All animales were housed, five per cage, in a room with a 12 hour light–12 hour dark cycle, and had free access to dry food and tap water, except as noted below.

Procedure. Virgin females were placed with potent males and examined every 12 hours for the presence of vaginal plugs; the day a vaginal plug was detected was designated as day 1 of pregnancy. Each pregnant female was randomly assigned to one of the various treatment groups. Alcohol treatment consisted of an intraperitoneal injection of 0.030 ml. per gram of body weight of a 25 per cent (v/v) solution of 95 per cent ethyl alcohol in physiologic saline.* Control animals

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received intraperitoneal injections of 0.030 ml. per gram of body weight of physiologic saline*. All injection were given between 9:00 and 10:00 A.M. The dosage of alcohol was hypnotic; the females remained comatose for up to 4 hours following treatment. Since the alcohol-treated animals were unable to eat or drink during this interval, all control animals were food and water deprived for 6 or 7 hours following saline treatment. All females were checked and weighed daily from the day of injection until they were killed on gestation day 18. Following death of the mother, the uterus was removed and the number and location of live fetuses, dead fetuses, and resorptions were recorded. Live fetuses were examined for external malformations, blotted dry, weighed to the nearest 0.1 Gm. and fixed in 10 per cent formalin.

Three experiments were done. In experiments 1 and 2 groups of female mice were treated on both of gestation days 8 and 9 or gestation days 10 and 11. The females used in experiment 1 were between 130 and 147 days old and those in experiment 2 were 70 (±3) days of age. Fetuses in experiments 1 and 2 were only examined for the presence of external malformations.

Experiment 3 was designed to provide information concerning the effects of a single treatment of alcohol. Groups of mice received either alcohol or saline on only one of gestation days 7 through 12. Females used in this experiment were 82 or 83 (±3) days of age when mated. In addition to external examination, fetuses were free-hand sectioned[10, 11] following fixation to permit detection of soft-tissue anomalies.

In all experiments fetal abnormalities were recorded and photographed.

Results

There was no maternal death attributable to the alcohol treatments in any experiment. Table I summarizes the results of experiments 1 and 2. These data indicate that the fetal mortality rate, which includes both dead fetuses and resorptions, was substantially elevated following alcohol treatment on either gestation days 8 and 9 or days 10 and 11. The frequency of live fetuses with externally visible malformations was also increased in the alcohol-treated groups, especially among the fetuses of the younger females. Exencephaly was observed in two fetuses from females treated with alcohol on days 8 and 9, whereas all other malformed fetuses had ectrodactyly.

On the basis of these data a more detailed study of the effects of alcohol on prenatal development in mice was undertaken. The results of experiment 3, in which groups of mice were treated on one of gestation days 7 through 12, are summarized in Table II and Fig. 1. It is clear that the fetal mortality rate was substantially increased following alcohol treatment on gestation days 9, 10, 11 or 12. A marginally significant elevation in fetal death occurred following alcohol treatment on day 8. The incidence of fetal anomalies was significantly increased following alcohol treatment only on days 8, 9, or 10. The type and incidence of the most commonly observed malformations are indicated in Table III. With the exception of hypoplastic atria, there was little overlap in the type of anomalies observed in the experimental and control fetuses. There was considerable time dependancy in both the overall teratogenic response to alcohol (Fig. 1), and in the genesis of specific malformations (Table III). Coloboma of the iris and ectrodactyly of the forepaws, the most frequently observed abnormalities, appeared to have critical periods around days 8 to 9 and day 10, respectively. With the possible exception of hydronephrosis and exencephaly, critical periods for other abnormalities were not apparent (Table III).

Although there was a slight trend toward decreased weight among both the normal and abnormal alcohol-

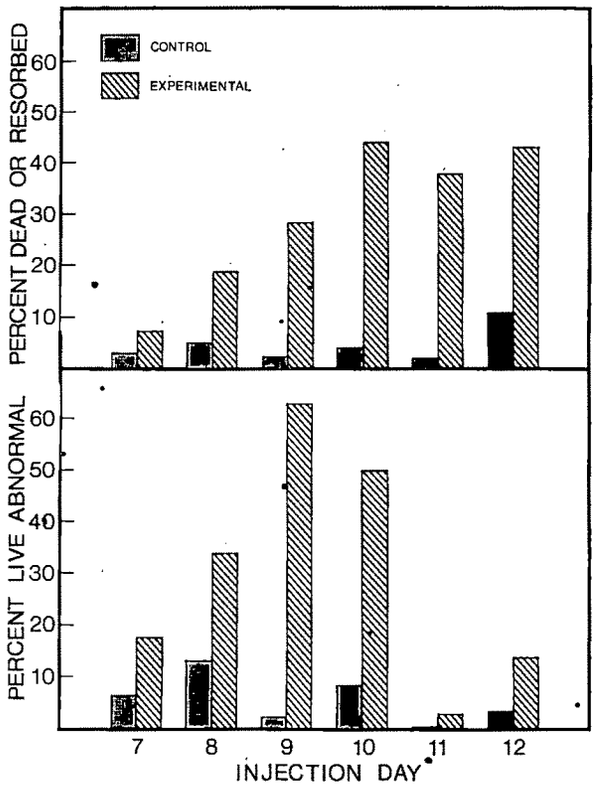


Fig. 1. The relationship of the percentage of dead and resorbed fetuses (fetal death) and the percentage of abnormal live fetuses to the day of treatment in experiment 3.

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Table I. Summary of experiments 1 and 2: Effect of treatment on gestation days 8 and 9 and gestation days 10 and 11

Treatment group	*Maternal age (days)*	*No. of litters*	*No. of implantations*	*Fetal mortality rate (%)*	*External malformations live (%)*
Alcohol	130-147	4	42	38.1	3.8
days 8 and 9	70	4	34	35.3	27.0
Saline	130-147	4	37	5.4	0
days 8 and 9	70	4	35	5.7	0
Alcohol	130-147	2	20	40.0	16.4
days 10 and 11	70	3	26	73.1	57.1
Saline	130-147	3	41	0	0
days 10 and 11	70	4	34	11.8	0

Table II. Summary of experiment 3: Effect of a single treatment on one of gestation days 7 to 12

Treatment		*No. of litters*	*No. of implantations*	*Fetal deaths*		*Live malformed*	
Day	*Group*			*No.*	*Statistical significance**	*No.*	*Statistical significance**
7	Alcohol	7	68	5	N.S.	11	N.S.
7	Saline	5	34	2		2	
8	Alcohol	9	91	17	$p < 0.05$	25	$p < 0.025$
8	Saline	4	40	2		5	
9	Alcohol	10	95	27	$p < 0.001$	41	$p < 0.001$
9	Saline	5	50	1		1	
10	Alcohol	9	93	42	$p < 0.001$	26	$p < 0.001$
10	Saline	5	51	2		4	
11	Alcohol	7	58	22	$p < 0.001$	1	N.S.
11	Saline	5	53	1		0	
12	Alcohol	6	51	22	$p < 0.001$	4	N.S.
12	Saline	4	38	4		1	

*Individual chi-square tests were used to compare alcohol to saline control for each day of treatment. N.S. indicates no significant difference.

Table III. Experiment 3: Frequency of various abnormalities as a function of the day of treatment

Treatment		*No. of live fetuses: abnormal/total*	*Number with abnormality*					
Day	*Group*		*Coloboma of iris*	*Ectrodactyly forepaw*	*Hypoplastic atria*	*Hydronephrosis*	*Exencephaly*	*Other*
7	Alcohol	11/63	8	0	2	0	1	2
7	Saline	2/32	0	0	0	0	0	2
8	Alcohol	25/74	17	1	1	0	2	5
8	Saline	5/38	2	0	3	0	0	1
9	Alcohol	41/68	38	0	0	2	1	7
9	Saline	1/49	1	0	0	0	0	0
10	Alcohol	26/51	9	13	1	4	0	3
10	Saline	4/49	3	0	0	0	0	1
11	Alcohol	1/36	0	0	0	0	0	1
11	Saline	0/52	0	0	0	0	0	0
12	Alcohol	4/29	2	2	1	0	0	1
12	Saline	1/34	1	0	0	0	0	0
Total	Alcohol	108/321	74	16	5	6	4	19
Total	Saline	13/254	7	0	3	0	0	4

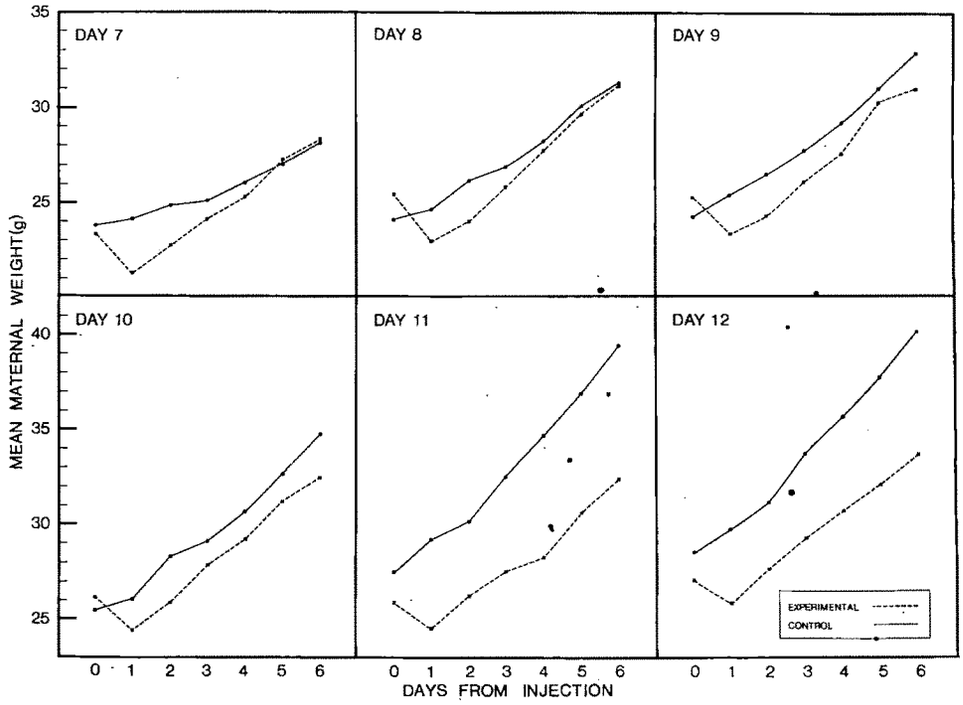


Fig. 2. Mean maternal body weight on each of the 6 days following treatment in experiment 3. The day of treatment is indicated in the upper corner of each box.

treated fetuses, they were not significantly lighter than control fetuses. The mean maternal body weights for the 6 days following injection are illustrated in Fig. 2. Alcohol-treated females lost between 1 and 2 Gm. in the 24 hours following injection, but thereafter gained weight at a rate comparable to controls. By the sixth day following injection, only females treated on day 11 or day 12 were significantly lighter than their respective controls (t test, two-tailed).

Comment

In summary, the data reported indicate that ethyl alcohol, in the dosage employed, is fetotoxic when administered to B6D2F$_1$/J mice on both of gestation days 8 and 9 or days 10 and 11, as well as in single doses on one of days 8 through 12. In addition, the frequency of fetal malformation was significantly increased when females were treated with single doses of alcohol on one of gestation days 8, 9, or 10. The malformations most frequently observed were coloboma of the iris and ectrodactyly involving only the forepaws. These malformations are of interest in light of the reports of eye[2–5, 7] digital, and palmar[2, 3, 5] abnormalities among the children of chronic alcoholic women. No other defects reminiscent of those reported in the fetal alcohol syndrome were consistently observed in these experiments, although there was a trend toward decreased weight in the alcohol-treated fetuses. The marked maternal weight loss following alcohol treatment, which was not observed in the food- and water-deprived controls, could be partly a result of nutritional disturbances, a frequent problem in the pregnant alcoholic patient.

Sandor et al[12–14] have reported that ethanol administered during organogenesis resulted in teratogenesis and *in ovo* death in the chick and prenatal death and growth retardation in the rat. It is clear that ethanol, in high doses at least, can disrupt prenatal development in at least three animal species. These data, in conjunction with the clinical reports of maternal alcoholism and offspring morbidity, provide a strong argument for further study of the effect of alcohol on development. Carefully controlled prospective epidemiologic studies are required to define the relationship in man, if any, between maternal alcohol ingestion and fetal damage. It may be prudent to follow children in such studies during the first years of life in order to evaluate psychomotor and neurologic development. In addition, further work with pregnant animals may be informative, including studies of lower dosage alcohol treatment and chronic alcohol dependancy.[15, 16]

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Addendum

Chernoff[17] has recently reported that mice consuming a liquid alcohol diet prior to and throughout pregnancy had increased rates of both fetal death and malformation. Skeletal, cardiac, and neural anomalies were detected, and both the teratogenic and fetotoxic effects appeared to be related to the amount of alcohol consumed (percentage of total calories derived from alcohol).

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Abnormal coagulation and fibrinolysis in eclampsia

A clinical and laboratory correlation study

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This study evaluated the existence of abnormally increased coagulation and fibrinolysis in 33 severely toxemic and eclamptic women by means of a combined hematologic profile with clinical and morphologic correlations. The dominant findings were: different degrees of thrombocytopenia, abnormal levels of blood fibrinogen, prolonged thrombin time, and positive protamine sulfate test. Altered activated partial thromboplastin time and positive ethanol gelation test were slightly less frequent, and only few cases showed prolonged prothrombin time or early lysis of euglobulins. These abnormalities seemed to be more numerous and more pronounced in the worst cases of the series and their severity seemed to be associated with the age of the patient and the presence of previous underlying disease. These variously handicapped pregnant women exhibited worse hematologic abnormalities, fell rapidly into decompensated intravascular coagulation, and provided most of the fatal cases in the series. Finally, the main findings were discussed and commented upon.

EVIDENCE LINKING an abnormally increased process of coagulation and fibrinolysis in the pathologic physiology of the toxemias of pregnancy has been accumulating in the last 25 years, after Schneider,[20] McKay and associates,[14] and Pritchard and associates[17] renewed interest on the subject. However, since most moderate and uncomplicated severe toxemia cases, and some eclamptic patients, do not seem to present this process, doubts over its universality still exist.[8, 12] Furthermore, before Henderson and associates[11] and Bonnar and associates[2, 3] were able to measure in these cases the products of fibrinogen-fibrin degradation finding conclusive evidence of disseminated intravascular coagulation in toxemic women was a difficult task.

The very nature of the coagulation and fibrinolysis process in normal conditions and in the early part of chronic abnormalities, such as may occur during pregnancy, seems to be elusive and conflictive. This results from its dynamic character that allows the interposition of compensatory mechanisms that insure a clinical and physiologic equilibrium in spite of the presence of important regional and systemic derangements of the coagulation-fibrinolysis process. Furthermore, a hypothetical state of chronic regional coagulation-fibrinolysis occurring in the placental intervillous circulation could not be detected until the magnitude of the local changes affected the systemic circulation and overcame the compensatory capacities of the liver, the bone marrow, and the reticuloendothelial system.[10, 15] In the end of such sequence of events, the significance of the local origin could be easily overshadowed by the fluorescence of the generalized phenomena.

Thus, if the differences in the basic pathophysiology between a mild and a very severe case of toxemia of pregnancy are mostly quantitative, even in instances of superimposed toxemia that may have qualitatively different predisposing factors, it may be postulated that the obvious abnormalities that are usually present in the severe cases also exist in the milder ones, but due

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Table I. Individual clinical and laboratory (hematology) profiles from 33 toxemic patients

Case No.	Age	Para	Weeks	BP diastolic (mm.)	Urine proteins (Gm./L.)	Diagnosis*	Type of delivery	Blood sampling		Died	Platelets	Fibrinogen	PT
								pre DEL (hr.)	post DEL (hr.)				
1	15	0	39	110	21.0	AE	C	2.5					
2	16	0	42	110	0.3	AE	C		3.0			U†	U
3	17	0	39	110	3.0	T	C		34.0		↓†	↑↑	
4	17	2	40	95	2.8	PE	V		36.0				
5	18	0	42	110	3.0	AE	C	6.0				↑	
6	18	0	33	120	1.0	AE	C	3.0					
7	19	0	42	110	1.0	AE	C	4.0				↑	
8	19	0	40	140	1.0	AE	C	2.5		*†	↓		
9	21	1	36	130	5.4	T	V	1.5				↓	
10	21	2	29	140	1.0	AE	V	9.0		*	↓		
11	22	0	35	110	28.0	T	C		3.0				
12	22	0	35	120	3.0	AE	C	2.0				↑	
13	22	0	33	140	3.0	AE	C	2.0					
14	23	3	44	120	5.0	PE	V		10.0		↓	↓	↑
15	23	0	35	110	10.0	T	C	28.0			↓		
16	24	1	32	120	3.0	T/H	V	9.0					
17	25	0	41	100	0.3	T	C		9.0			↓	
18	26	2	31	100	3.4	IE	C	22 days				↑	
19	27	1	39	110	0.4	T	V	69.0			↓		
20	27	0	22	130	3.0	T/H	IU	44.0		*		U	
21	27	6	34	100	1.8	T	V	25.0					
22	29	4	32	150	3.0	T/H	C	1.0		*	↓	↓	
23	32	4	27	170	9.0	PE/H	C		44.0	*			
24	32	4	34	120	3.0	AE/H	C		8.0		↓	↓	↑
25	32	2	23	140	1.0	AE/H	C	4.0		*			
26	33	2	39	130	3.0	PE	V		9.0	*	↓	↑	
27	34	5	35	110	3.0	T/H	V	14.0				↑	
28	34	6	35	110	0.6	T	V		45.0		↓		U
29	34	1	35	170	7.7	T/H	C	2.5			↓	↑	
30	39	10	40	90	0.7	PE	V		10.0		↓	↓	
31	39	4	41	90	1.0	PE/H	C		11.0		↓	↓	
32	41	0	36	110	0.3	T	C		0.5			0 ↓	↑
33	42	4	33	140	—	T	C		5.0	*	↓	0*↓	↑

*Abbreviations: T = toxemia; E = eclampsia; A = antepartum; P = postpartum; I = intercurrent; H = chronic hypertension; (
†↓ = Decreased; ↑↑, ↑ = increased; + = positive; U = unknown (not performed); 0 = nondetectable fibrinogen; * = maternal death

to methodological limitations, they have escaped confirmation.

The present study attempted to detect and evaluate the existence of abnormally increased coagulation-fibrinolysis processes in eclamptic and severely toxemic women, by means of a combined hematologic laboratory profile carried out as close to the critical period as possible. Because of the well-known clinical aspects and therapeutic imperatives of such cases, it was not practical to adhere to a very strict schedule of blood sampling in all patients. However, precautions were taken not to include cases where interference with the hematologic tests seemed likely.

Clinical data

A total of 33 toxemic patients were included in the present study; 18 with eclamptic convulsions, and 11 instances of severe and 4 of moderate toxemia of pregnancy. At least 9 of these patients had reliable records of toxemia in previous pregnancies and/or history of chronic hypertension, 5 had severe superimposed toxemia with one liver rupture, and 2 had postpartum and 2 antepartum eclampsia in the problem pregnancy. Eight patients died; half of these deaths took place in the smaller group of patients with previous underlying disease. There were six complete postmortem studies.

The average age of the patients was 26.4 years (S.D. 7.7). There were 8 teenagers and 11 women older than 30 years of age. Fourteen women were nulliparous and 10 had had three or more previous deliveries. The average gestational age in 27 cases was 35.5 weeks (S.D. 5.6). One patient was 22 weeks pregnant, another was 23 weeks, and 18 were 35 weeks or less (see Table I).

Three patients were admitted with recently dead fetuses, one patient died with a nonviable undelivered fetus, and the rest of the patients gave birth to live fetuses with 5 neonatal deaths due to nonviability.

PTT	*TT*	*Protamine*	*Ethanol*	*Eug. lysis*	*No. of altered tests*
	↑	+†			2
↑		+		U	2
		+			3
↓		+			2
	↑	+	+		4
↓	↑				2
↑	↑				3
	↑	+		+	4
		+	+		3
	↑↑†		+		3
↓		+	+	+	4
	↑				2
↑	↑				2
↑	↑	+			6
	↑				2
↓	↑				2
	↑↑	+			3
↓					2
↑	↓				3
↓		+		U	2
↓	↑	+	+		4
↓	↑↑	+		+	6
↑		+			2
↑		+	+	+	7
↑	↑	+	+		4
↑	↑↑				4
	↑	+			3
U	↑↑	+			3
					2
↑	↑	+			5
	↑↑			+	4
↑	↑↑	+	0†	U	5
↑	↑	+	0		6

= cesarean operation; V = vaginal delivery; IU = died undelivered.

Eleven patients delivered vaginally and 21 by cesarean operations. The toxemic condition on admission averaged 175.5 (S.D. 33.4) mm. Hg systolic and 120.2 (S.D. 20.0) diastolic, with 4.15 (S.D. 5.9) Gm. per liter of proteinuria (Table I).

Technical data

A total of 11.5 ml. of blood was taken through a nonheparinized central venous pressure catheter after discarding the first 10 ml., and was distributed as follows: 2.5 ml. in a plastic test tube containing 0.025 ml. of 10 per cent EDTA-Na solution for platelet counting, and 9.0 ml. divided in two plastic test tubes containing 0.5 ml. of 3.8 per cent sodium citrate solution each and centrifuged at 3,000 r.p.m. for 15 minutes. Immediately afterward the following analyses were carried out: platelet count,[4] blood fibrinogen,[7] prothrombin time,[19] celite-activated partial thromboplastin time,[18] thrombin time,[1] serial dilution protamine sulfate test,[9] ethanol gelation test,[5] and euglobulin lysis time.[6]

Results

Timing of blood sampling. Samples were taken within the first 6 hour period after admission in 12 cases, within the second 6 hours in 6 cases, within the third in three, and within the fourth 6 hour period in two cases. In the remaining 10 patients, blood was drawn after 1 to 26 days of hospitalization.

Blood sampling took place at an average of 9.4 hours before delivery time in 17 cases, and in two additional patients this occurred 2.8 hours and 22 days before delivery. In 10 cases blood was drawn at an average of 6.9 hours after delivery, and in 4 additional patients this took place between the first and second postdelivery days (Table I).

Platelets. Platelet count averaged 113,401 (S.D. 71,521) per cubic millimeter in the 33 patients of the study. This count was below 75,000 per cubic millimeter in 14 cases. There were no statistically significant clinical differences between these thrombocytopenic patients and the values for the whole group. However, the former were an average of 5.3 years older than the rest and accounted for five of the eight maternal deaths, seven of the 18 cases of eclampsia, and four of the 9 patients with previous underlying disease (Tables I and II).

Fibrinogen. A total of 30 cases averaged 288.7 (S.D. 171.2) mg. per cent of fibrinogen. This figure was below 200 mg. in 9 patients, including two cases with nonmeasurable fibrinogen, and it was above 400 mg. in eight patients. The low fibrinogen cases accounted for six of the 14 cases with platelets below 75,000 per cubic millimeter, but otherwise there was no apparent association between the level of fibrinogen and the platelet count in the remaining patients. The nine cases with low values of fibrinogen had no remarkable clinical differences from the rest, but although not statistically significant they were an average of 8.2 years older, and the two cases with nonmeasurable fibrinogen were 41 and 42 years of age, respectively (Tables I and II).

Prothombin time (PT). This test was performed in 31 patients averaging 13.7 (S.D. 2.3) seconds against 12.6 (S.D. 0.8) in the corresponding control plasmas, a difference of only 1.1 seconds. However, this PT was at least 4 seconds longer than its control in 4 instances (average 6.5 seconds). These cases of prolonged PT also presented thrombocytopenia below 75,000 in three instances, and fibrinogen below 200 mg. in the four cases. The cases also included one liver rupture that provided the only death in this small group. In

Table II. Complete individual hematologic profile in 33 toxemic cases

Case No.	Platelet count (cu. mm.)	Fibrinogen (mg. %)	PT		PTT		TT		Protamine	Ethanol	Eug. lysis
			Problem (sec.)	Control (sec.)	Problem (sec.)	Control (sec.)	Problem (sec.)	Control (sec.)			
1	143	270	11.8	12.0	35.0	38.0	22.5	15.5	+†	−	−
2	127	U†	U	U	59.0	45.0	16.0	15.0	+	−	U
3	63.5	460	13.2	13.4	47.0	46.0	13.0	13.8	+	−	−
4	230	330	12.4	13.0	33.0	47.0	19.0	19.0	+	−	−
5	142	460	12.2	12.8	27.5	27.0	29.0	18.0	+	+	−
6	135	380	11.5	13.4	27.0	33.0	22.0	16.5	−†	−	−
7	263	433	13.0	13.0	46.0	34.0	25.8	20.0	−	−	−
8*	75	275	13.5	12.8	43.5	40.0	21.8	14.8	+	−	+
9	220	183	11.2	10.5	45.0	45.0	21.0	18.5	+	+	−
10*	36	320	14.0	12.8	35.0	39.5	52.0	19.0	−	+	−
11	185	220	12.5	13.0	35.0	40.0	15.4	16.5	+	+	+
12	78	550	11.8	13.4	27.0	30.5	24.5	16.0	−	−	−
13	94	300	14.0	13.5	47.0	40.0	25.0	18.5	−	−	−
14	44	197	17.0	11.0	46.5	32.0	23.4	17.0	+	−	−
15	29	300	12.4	13.2	34.0	37.0	26.0	17.5	−	−	−
16	134	U	12.0	12.2	40.0	47.0	29.0	20.0	−	−	−
17	105	125	15.0	12.5	40.0	40.0	30.0	16.0	+	−	−
18	263	550	11.6	11.8	31.0	39.4	17.4	15.0	−	−	−
19	72	300	10.8	12.8	40.0	35.0	15.0	20.0	−	−	−
20*	290	U	13.2	13.2	30.0	39.0	20.0	16.8	+	−	U
21	98	220	13.2	13.0	34.0	42.0	25.0	18.0	+	+	−
22*	49	133	16.5	13.0	35.0	40.0	55.0	20.0	+	−	+
28*	160	370	12.2	12.4	43.0	34.0	20.5	18.0	+	−	−
24	30	140	18.8	11.8	55.0	36.8	13.4	15.0	+	+	+
25*	76	320	12.8	12.2	46.0	39.0	30.6	21.6	+	+	−
26*	23.7	433	14.0	12.5	41.0	36.5	55.5	18.5	−	−	−
27	153	590	16.0	12.2	43.0	39.3	20.5	14.5	+	−	−
28	70	240	U	U	U	U	29.0	14.0	+	−	−
29	49	495	11.8	13.4	35.4	33.0	19.5	17.2	−	−	−
30	55	150	15.5	13.2	57.0	48.0	23.5	16.0	+	−	−
31	56	116	11.9	11.0	27.4	27.0	55.0	23.0	−	−	+
32	125	0	18.0	12.0	72.0	33.0	45.0	21.0	+	0†	U
33*	69	0	20.0	13.0	103.0	35.0	20.5	16.0	+	0	−

*Maternal death.

†+ = positive; − = negative; U = unknown (not performed); 0 = nonmeasurable fibrinogen.

these patients, blood was collected after cesarean operation in three cases and after vaginal delivery in one (Tables I and II).

Activated partial thromboplastin time (APTT). This test was performed in 32 patits giving an average of 42.5 (S.D. 14.8) seconds against 38.1 (S.D. 5.4) in the corresponding control plasmas; a difference of 4.4 seconds. However, the APTT was at least 5 seconds longer than its control in 12 patients and at least 5 seconds shorter in eight. The group with prolonged APTT also exhibited thrombocytopenia below 75,000 in six instances and fibrinogen below 200 mg. per cent in four and above 400 mg. in two. Prolonged prothombin time occurred in four and accounted for four of the maternal deaths. The eight cases with short APTT were associated with one case of thrombocytopenia; one each for low and high fibrinogen levels. This group accounted for two maternal deaths (Tables I and II).

Thrombin time (TT). The problem plasma of 33 patients averaged 26.7 (S.D. 11.9) seconds against 17.5 (S. D. 2.3) in the control plasma, a difference of 9.2 seconds. One patient showed a shortened TT of 15.0 seconds against 20.0 in the control, and 22 patients showed thrombin times at least 4 seconds longer than their controls (average difference = 13.6 seconds). Seven patients showed thrombin times at least 15.0 seconds longer than the controls (average difference = 27.3 seconds). Five of these patients had platelet counts below 75,000 and the remaining two had 105,000 and 125,000 platelets per cubic millimeter, respectively. Four of the patients had fibrinogen levels below 200 mg. and the remaining three had 240, 320, and 433 mg. per cent. Activated partial thromboplastin time was prolonged in two, normal in three, short in one, and was not recorded in the last patient.

Protamine sulfate and ethanol gelation tests. A total of 21 patients gave positive protamine sulfate tests; six

Table III. Main clinical, hematological, and morphological data in the eight maternal deaths of the study

Case No.	Age	Diagnosis		Post-mortem hr.	Main cause of death	Periportal hemorrhagic necrosis	Glomercular toxemic lesion	Capillary	
		Clinical	Hematological					fibrin: kidneys	thrombi: lungs
8	19	AE	D	No	Brain hemorrhage†	–	–	–	–
10	21	AE	C	No	Brain hemorrhage†	–	–	–	–
20	27	T/H	Hy	3.0	Brain hemorrhage	No*	Intense	No	Yes
22	29	T/H	D	5.3	Brain hemorrhage	Marked	Intense	No	No
23	32	PE/H	D	1.0	Bronchopneumonia	Marked	Intense	No	Yes
25	32	AE/H	Hy	1.5	Brain hemorrhage	Yes	Intense	No	Yes
26	33	PE	Hy	1.0	Brain hemorrhage	Yes	Intense	No	No
33	42	T	D	2.2	Hepatic rupture	Marked	Intense	No	Yes

*Periportal degenerative changes only.

†Clinical diagnosis.

Abbreviations: A = Antepartum; P = postpartum; E = eclampsia; H = chronic hypertension; T = toxemia; C, Hy, D = compensated, hypercompensated, and decompensated intravascular coagulation, respectively.

of these gave also a positive ethanol gelation test, and only one negative protamine patient gave an ethanol positive test (Tables I and II).

Twelve patients had negative protamine sulfate tests. Their hematologic diagnoses were: compensated intravascular coagulation in six, hypercompensated in five, and decompensated in only one patient. Eight had low platelet counts, two were normal, and two had high normal values (Tables I and II).

For the first 21 patients of this study the 50 per cent ethanol solution was prepared from several days to 2 or 3 weeks before the test was actually performed. The solution was prepared immediately prior to the test in the remaining twelve patients, also. Six of the seven positive tests took place in the latter patients.

Euglobulin lysis time test. This test was positive within the first 90 min. in five patients of the series.

Interpretation of altered hematologic tests. The diagnosis of disseminated intravascular coagulation seemed justified in all the patients of the present study. Using the fibrinogen level as the basic indicator of compensation, hypercompensation, or decompensation in the abnormal process of coagulation-fibrinolysis,[16] and the remaining laboratory and clinical data as complementary and confirmatory of the hematologic diagnosis, the condition of the 33 patients of the study was interpreted as follows; compensated intravascular coagulation in 11 patients, hypercompensated in 9, and decompensated in 13. However, there was no parallelism between platelet function and fibrinogen levels, since there were six thrombocytopenic patients in the compensated group, four patients with normal platelet counts in the decompensated group, and five normal and four thrombocytopenic patients in the hypercompensated intravascular coagulation group.

Similarly, there was no correlation between the state of compensation, hypercompensation or decompensation, and the length of gestation, the type of delivery, the blood pressure, amount of proteinuria, or number of convulsions. But, on the other hand, the average age of the compensated group was 21.9 ± 1.6 years, while it was 28.6 ± 1.66 in the remaining patients ($P < 0.01$), and consequently eight of the nine patients with the clinical diagnosis of underlying disease belonged to this latter group. Also, the compensated group had only one maternal death, while there were three in the hypercompensated and four in the decompensated group.

Postmortem findings. Eight patients died and six had complete postmortem studies. The toxemic glomerular lesion was found in the six cases, periportal hemorrhagic necrosis was present in all but one, four cases had fibrin thrombi in the lungs' capillaries, and large parenchymatous brain hemorrhages were confirmed in four autopsies and in the two patients without postmortem study by clinical data alone (Table III).

Comment and conclusions

The toxemic patients included in the present study were those cases severe enough to require admittance to the intensive-care unit, and arriving at a time and with the clinical characteristics compatible with the performance of the hematologic tests during the period of maximum laboratory efficiency. Thus, from this point of view, the patients became a special group, but in no other way were they premeditatedly selected. Most of the mortality cases were probably unusually ill women, but the group of survivors can be taken as a fair representative of an average toxemic population.

This study did not include a control group because of the wide dissimilarities of the problem cases, that

would have required not one but various matched control groups in age, parity, amenorrhea, type of delivery, and timing of blood sampling. Because of this, the tests were given wider margins of normality than those commonly accepted in the literature and all the important individual information was presented.

In spite of the wide variability of the clinical and laboratory data, several general tendencies could be demonstrated. The dominant findings were: different degrees of thrombocytopenia, abnormal levels of blood fibrinogen, prolonged thrombin time that remained abnormal in spite of dilution with normal plasma, and positive protamine sulfate test. Altered activated partial thromboplastin time and positive ethanol gelation test were slightly less frequent and only few cases had prolonged prothrombin time or early lysis of euglobulins. The abnormalities of the coagulation-fibrinolysis process, estimated by the above mentioned tests, seemed to be slightly more numerous and more pronounced in the worst cases of the series. This severity seemed to be somewhat associated to the age of the patient and to the presence of previous underlying disease.

The dynamics of disseminated intravascular coagulation imply the continuous interplay of several simultaneous phenomena. However, in spite of this essentially simultaneous character, some processes dominate others in apparent succession, giving rise to various basic stages that may follow a subtle course or an abrupt one. The dominant phase in an abnormal coagulation-fibrinolysis process at a given moment depends on the duration and intensity of the noxious primary cause, that in turn depends on other pathogenic factors, and of the homeostatic compensatory capacities of the reticuloendothelial system, the liver, and the bone marrow. These factors and mechanisms vary considerably from case to case and may not follow parallel directions in the same patient. Thus, when a hematologic profile is carried out, the results will show the dominant tendency at that precise moment, but, excepting some very definite situations, they will be less accurate in predicting the future pattern. Furthermore, the interaction of physiologic variables that elude quantitation and the influence of the therapy directed toward the basic problem affect the prognosis of the hematological condition.

Our study confirmed to a very large extent most of the findings and interpretations of previous workers on the subject, and it also emphasized the influence of advancing age and of underlying pathology in the severity and final outcome of these cases.[13] Thus, at present, it seems logical to assume that women with any type and degree of previous underlying disease predisposing to toxemia of pregnancy, experience a bipolar phenomenon. For one part, these previous defects increase the importance of the whole pathogenic complex, and for another one, they reduce the compensatory capacities of many essential homeostatic mechanisms, such as the bone marrow, the liver, and the reticuloendothelial system that are particularly important in regards to an abnormal process of chronic coagulation-fibrinolysis. These variously handicapped pregnant women may exhibit worse hematologic abnormalities, fall rapidly into decompensated disseminated intravascular coagulation, and provide most of the fatal cases in a toxemic population.

However, in spite of the importance of chronic and acute disseminated intravascular coagulation in the pathologic physiology of the toxemias of pregnancy, this does not seem to be the final answer to the problem. The available pieces of the jigsaw puzzle point toward a multifactorial pathogenesis, and if this turns out to be the correct perspective, the increased process of coagulation and fibrinolysis will be identified as an important link in the rather long chain of reciprocal events forming a variable pathogenic complex that manifests itself clinically through limited common channels that end in the toxemic syndrome.

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Placental transfer of clindamycin and gentamicin in term pregnancy

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The pharmacokinetics of clindamycin and gentamicin were studied in women given these antibiotics prior to cesarean section. Maternal clindamycin levels were within the normal range and cord levels were within the therapeutic range for this antibiotic. For gentamicin, however, maternal levels were depressed, with a concurrent depression of cord levels. This may have significant implications for the use of gentamicin in maternal and fetomaternal infections.

BECAUSE of the frequent use of antibiotics during pregnancy for maternal infections (such as pyelonephritis and pneumonia), for fetomaternal infections (such as chorioamnionitis), and for prophylaxis, knowledge of the placental transfer of these agents is essential. Clindamycin and gentamicin have found widespread use in pregnancy. Clindamycin provides good coverage of gram positive organisms and excellent coverage of Bacteroides species, which have recently been emphasized as major pathogens in pelvic infection.[1, 2] Gentamicin offers optimal gram-negative coverage.

There is, however, little information on the placental transfer of these two antibiotics. In the present study, antibiotic concentrations in the mother, cord, and neonate were assayed in cesarean section patients receiving a perioperative course of clindamycin and gentamicin for prophylaxis.[3]

Materials and methods

Fifty-four patients undergoing cesarean section on the Obstetrical Service of the Hospital of the University of Pennsylvania were given clindamycin in a dose of 600 mg. (5.5 to 11.1 mg. per kilogram) intravenously and gentamicin in a dose of 80 mg. (0.6 to 1.4 mg. per kilogram) intramuscularly 30 minutes before and 8 hours after surgery. Maternal serum levels of both antibiotics were measured at intervals from 15 minutes to 8 hours after the first dose. Neonatal serum antibiotic levels from capillary tube samples were measured at intervals from 1½ to 6 hours after injection. At delivery, amniotic and umbilical venous blood antibiotic levels were determined. An agar diffusion method of antibiotic assay was utilized,[4] employing a strain of alpha-hemolytic streptococcus to measure clindamycin levels and a strain of *Escherichia coli* to measure gentamicin levels. Informed consent was obtained to draw blood samples.

Results

Fig. 1 shows clindamycin concentrations in the maternal blood after a 600 mg. intravenous dose. This regression curve, derived by computer analysis of 58 maternal samples, may be expressed as the equation: $y = 6.813 - 0.0386(x) + 0.0006(x)^2$, where y = antibiotic concentration in micrograms per milliliter and x = time in minutes after injection. Since no blood samples were obtained within the first 15 minutes after injection, this expression is not valid during this period. The curve closely simulates that seen in nonpregnant individuals,[5] with peak serum concentrations of 6 to 9 μg per milliliter and a gradual decline over 6 to 8 hours. Fig. 2 demonstrates the cord blood concentration of clindamycin. This curve was derived from 42 umbilical cord vein samples and may be expressed

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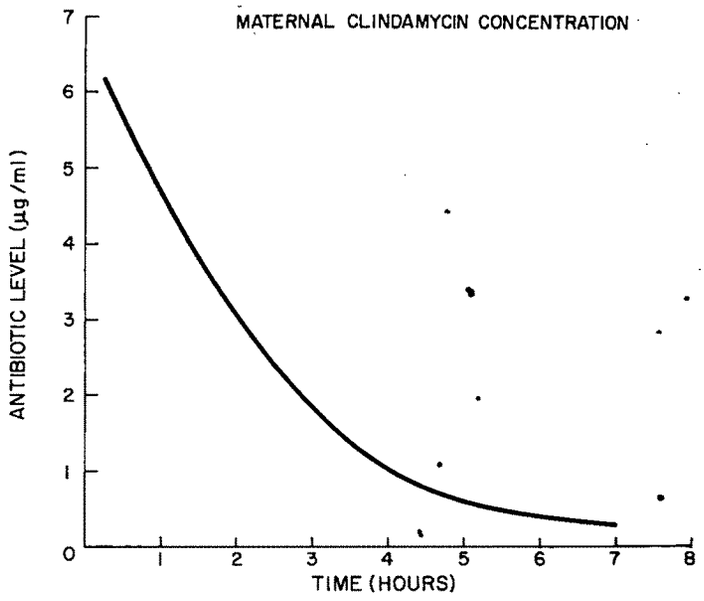


Fig. 1. Maternal clindamycin concentration after a 600 mg. intravenous dose.

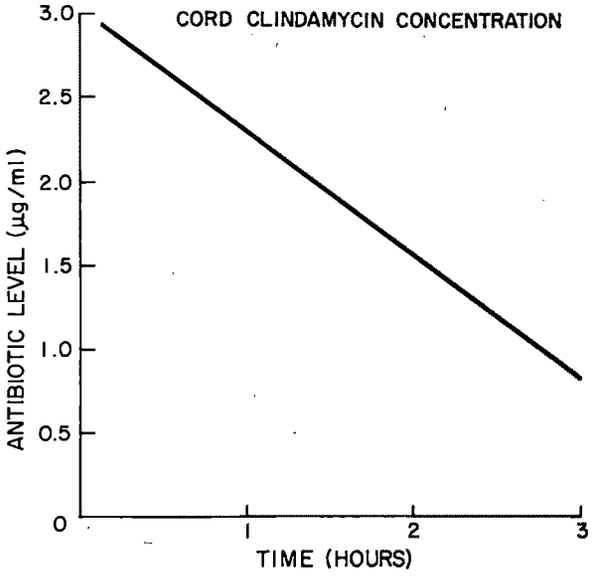


Fig. 2. Cord venous clindamycin concentration after a 600 mg. intravenous dose to the mother.

by the equation: $y = 3.004 - 0.0120(x)$. Peak cord concentrations of clindamycin were approximately 3 μg per milliliter, occurring at about 20 minutes following injection. Maximum cord blood concentrations were 46 per cent of simultaneous maternal levels.

Fig. 3 depicts gentamicin concentrations in the maternal blood following an 80 mg. intramuscular dose. Derived from 58 maternal venous samples, this curve may be expressed as: $y = 2.214 - 0.00891(x) + 0.00001(x)^2$. Maximum levels of gentamicin were approximately 2 μg per milliliter. Significantly, these levels are fully 50 per cent lower than those observed in nonpregnant individuals.[6] In a subsample of patients, the per cent urinary excretion of gentamicin was measured and found to be similar to that seen in nonpregnant individuals, 50 to 90 per cent of the dose.[6] Fig. 4, showing the cord blood concentration of gentamicin, was derived from a wide scatter of 30 points by averaging samples in 30 minute intervals. The maximum cord blood levels were 42 per cent of the simultaneous maternal blood levels. Although the cord levels were relatively high when compared to the maternal concentrations, the absolute levels, with peaks of less than 1 μg per milliliter 1 hour after the injection, were extremely low.

Gentamicin measured in 25 neonatal venous blood specimens during the first 6 hours of life was consistently below 1 μg per milliliter. Clindamycin levels in the same specimens were below 2 μg per milliliter. Neither antibiotic was detected in nine amniotic fluid samples obtained between 30 and 60 minutes after injection.

Comment

It is essential to determine the pharmacokinetics of antimicrobial agents in pregnancy because of their use in the treatment of infection and in the preoperative prophylaxis of infection. Clindamycin and gentamicin have activity against a majority of the microorganisms isolated from the normal female genital tract.[1, 2] Few

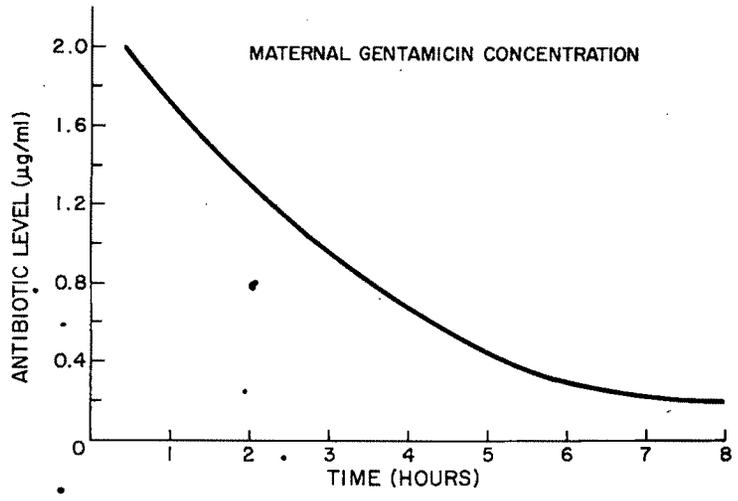


Fig. 3. Maternal gentamicin concentration after an 80 mg. intramuscular dose.

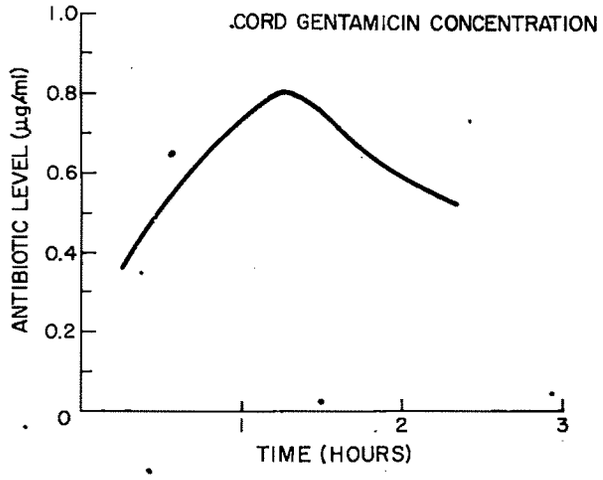


Fig. 4. Cord venous gentamicin concentration after an 80 mg. intravenous dose to the mother.

studies have been reported on the placental transfer of either of these agents. In one study gentamicin was shown to reach peak cord levels equivalent to 33 per cent of maternal levels, within 1 hour following intramuscular injection.[7] However, this investigation was limited to low doses (approximately one half the current standard) and did not include levels of the drug in the fetus or the mother after birth. One study of clindamycin placental kinetics is available but deals only with midtrimester therapeutic abortion patients who received oral clindamycin.[8] Lincomycin, from which clindamycin was derived, has been studied[9] but differences in structure, absorption and activity make it difficult to apply this data to clindamycin.

In this study involving 54 women undergoing cesarean section, maternal serum gentamicin levels were consistently lower than those observed in nonpregnant individuals, a phenomenon previously observed with kanamycin.[10] Increased renal clearance did not appear to be responsible for such finding. Possibly, the increased plasma volume of pregnancy or a possible inactivation of gentamicin by clindamycin[11] may have been responsible for these results. Serum clindamycin concentrations in these pregnant women at term were found to be equal to those in nonpregnant individuals.[5]

Cord concentrations of gentamicin were 42 per cent of simultaneous maternal levels while cord clindamycin concentrations were 46 per cent of maternal levels, and within the therapeutic range for this antibiotic.[5] For gentamicin, however, because of lowered maternal levels, these concentrations were, in the majority of instances, below the therapeutic range for this agent.[6]

Gentamicin and clindamycin were not detectable in the amniotic fluid during the first hour after injection. However, it has been demonstrated with other antibiotics that therapeutic levels may not be achieved in the amniotic fluid for at least 4 to 6 hours.[9, 10]

The antimicrobial effect of such a prophylactic antibiotic regimen is discussed elsewhere.[3] However, it is important to note that the significant reduction in maternal and cord gentamicin concentrations may have important therapeutic implications in pregnant women at term given standard doses of gentamicin in combination with clindamycin.

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Erythrocyte 2,3-diphosphoglycerate in normal and hypertensive gravid women and their newborn infants

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Red blood cell 2,3-diphosphoglycerate (2,3-DPG) levels were studied in 227 normal gravid women in all stages of pregnancy, active labor, and early post partum. An increase to peak levels was observed at 19 to 22 weeks followed by a gradual fall toward normal. During labor and early post partum, 2,3-DPG again rose significantly above levels observed at term. Serial 2,3-DPG values through the third trimester, labor, and postpartum period in 11 hypertensive mothers and 20 normal gravid control subjects and their newborn infants were studied. Maternal 2,3-DPG levels were significantly higher in the hypertensive mothers when compared to control mothers at similar gestational stages. However, no difference in 2,3-DPG levels was observed when newborn infants of hypertensive mothers were compared with those of normal control subjects.

THE POSITION OF the oxygen dissociation curve of fetal blood to the left of the maternal curve is considered an important factor in facilitating placental transfer of oxygen. One of the most important regulators of the position of this curve is the intracellular concentration of 2,3-diphosphoglycerate (2,3-DPG) in the erythrocytes.[1] In binding with the hemoglobin molecule, 2,3-DPG influences the oxygen affinity of hemoglobin. As a result, increased levels of 2,3-DPG facilitate oxygen unloading from the red cells to peripheral tissues, which is expressed in a shift to the right of the oxygen dissociation curve.[2]

An increase of 30 per cent in 2,3-DPG concentration has been described at term pregnancy.[3] This increase has been interpreted to reflect a compensatory mechanism to facilitate oxygen release in the presence of "functional anemia" during pregnancy. Such compensatory elevations, due to hypoxia, have been described in a variety of clinical conditions.[4–7] In pregnancy, hypertension, either chronic or in the form of pre-eclampsia, has been associated with generalized vasoconstriction, most pronounced by a 60 per cent diminution in uterine placental flow.[8]

Maternal hypertension is often associated clinically with intrauterine growth retardation of the fetus.[9, 10] Recently, lower than normal 2,3-DPG levels were reported in a group of fetally malnourished newborn infants. The authors theorized that this shift to the left

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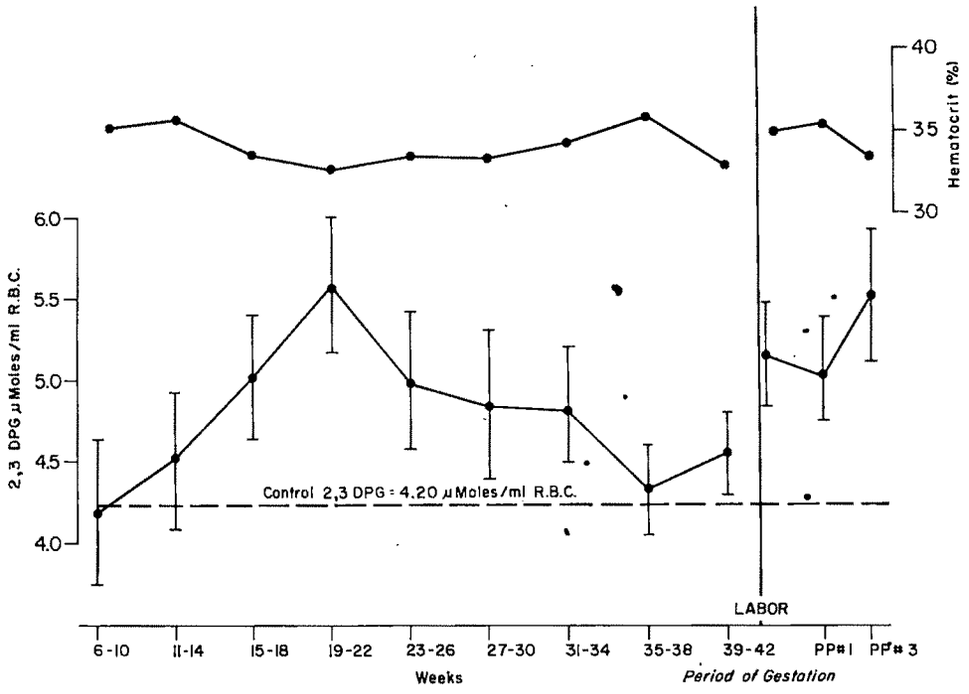


Fig. 1. Graphic display of 2,3-DPG and hematocrit values in normal pregnancy, during labor, and immediately post partum.

in the fetal dissociation curve could be a compensatory mechanism to overcome intrauterine hypoxia.[11]

In this report, results are presented of detailed analysis of erythrocyte 2,3-DPG levels throughout pregnancy, labor, and the postpartum period in normal gravidas.

Serial follow-up of hypertensive gravid women and their newborn infants was made and compared with follow-up of normal gravid control women.

Material and methods

The normal range of values throughout pregnancy was determined by studying single samples obtained from 163 gravid women attending the antenatal clinic at the Nassau County Medical Center. Ages of the women ranged between 16 and 39 years; gestational ages were between six and 42 weeks as determined by the last menstrual period and corresponding uterine size. All patients had a normal course of pregnancy at the time of study. Patients with doubtful gestational age or anemia as evidenced by a hematocrit below 30 per cent were excluded. In addition, 24 single specimens were obtained from patients during the active phase of term labor; 26 specimens were obtained on the first postpartum day, and 15 samples were taken on the third postpartum day.

Eighteen single blood specimens were also obtained from patients with hypertensive pregnancies after 34 weeks of gestation, in active labor, and early post partum. Hypertension was defined as blood pressure of at least 140/90 on two separate occasions.

Blood was drawn from the antecubital vein into a heparinized tube. One milliliter of whole blood was immediately pipetted into 3.0 ml. of 8 per cent cold trichloracetic acid. The specimens were then centrifuged for 10 minutes at 3,000 r.p.m. The 2,3-DPG in the supernatant was determined by a coupled enzymic method following the oxidation of reduced nicotinamide adenine dinucleotide at 340 nm. with a spectrophotometer.*[12] Hematocrit values were determined by a microcapillary centrifuge technique.

The results were expressed in micromoles of 2,3-DPG per milliliter of red blood cells.

Twenty patients with normal gestations and eleven hypertensive patients attending the prenatal clinic volunteered for serial follow-up from the thirty-fourth week of gestation through labor and postpartum Days 1 and 3. A minimum of four maternal specimens was studied for each patient. Mixed cord blood was obtained at the time of delivery from the newborn

*Model DU, Beckman Instruments, Inc., 2500 Harbor Blvd., Fullerton, California 92634.

Table I. 2,3-DPG in normal pregnancy

Weeks of gestation	Normotensive patients				Hypertensive patients			
	No.	Hct. (%)	2,3-DPG (μmoles/ml. of red blood cells)	Significantly higher than nonpregnant control value	No.	Hct. (%)	2,3-DPG (μmoles/ml. of red blood cells)	Significantly higher than parallel normotensive value
6-10	12	35.2 ± 2.4	4.22 ± 0.66	N.S.		—	—	—
11-14	11	36.2 ± 3.41	4.51 ± 0.69	N.S.		—	—	—
15-18	16	33.9 ± 2.4	5.08 ± 0.57	*		—	—	—
19-22	14	33.0 ± 2.3	5.58 ± 0.61	†		—	—	—
23-26	12	33.8 ± 3.0	4.96 ± 0.66	‡		—	—	—
27-30	11	33.6 ± 2.8	4.86 ± 0.69	§		—	—	—
31-34	19	34.4 ± 2.9	4.82 ± 0.53	‡		—	—	—
35-38	33	36.2 ± 3.5	4.33 ± 0.39	N.S.	3	36.3 ± 3.1	6.83 ±	†
39-42	35	32.9 ± 3.4	4.56 ± 0.39	N.S.	8	37.2 ± 3.7	6.10 ±	
Labor	24	35.0 ± 4.0	5.22 ± 0.45	†	3	34.0 ± 3.0	5.3 ±	
Postpartum Day 1	25	35.4 ± 3.7	5.03 ± 0.45	†	4	37.0 ± 4.0	6.3 ±	§
Postpartum Day 3	15	33.8 ± 3.6	5.53 ± 0.59	†		—	—	

N.S. = Not significant.
*Significant to P = 0.01 by the two-tailed t test.
†Significant to P = 0.001 by the two-tailed test.
‡Significant to P = 0.05 by the two-tailed t test.
§Significant to P = 0.1 by the two-tailed t test.

Table II. 2,3-DPG levels in normal and hypertensive maternal-newborn pairs

	Normotensive pregnancies			Hypertensive pregnancies			Significance of t test for 2,3-DPG difference between normal and hypertensive groups
	Hct. (%)	2,3-DPG (μmoles/ml. of red blood cells)	No.	Hct. (%)	2,3-DPG (μmoles/ml. of red blood cells)	No.	
Mothers							
Prelabor (34-42 weeks of gestation)	34.5 ± 3.1	4.59 ± 0.98	19	35.5 ± 3.7	5.85 ± 0.73	10	P = 0.001
Labor—active phase	35.4 ± 2.6	5.02 ± 0.98	16	35.4 ± 3.1	5.48 ± 1.05	9	N.S.
Postpartum (0-3 days)	36.2 ± 2.6	5.10 ± 1.13	16	32.7 ± 2.9	5.92 ± 1.60	9	N.S.
Babies							
At delivery (cord blood)	47.0 ± 2.1	4.44 ± 1.02	17	46.7 ± 3.9	4.6 ± 1.27	11	N.S.
On third day (heel stick)	48.1 ± 2.3	5.04 ± 0.93	15	43.4 ± 4.7	5.52 ± 2.31	5	N.S.

N.S. = Not significant.

infant; a second specimen was obtained from the baby by heel stick on the third day of life. Hematocrits and 2,3-DPG values were assessed as above. Birth weights were recorded, and the baby's weight percentile was determined.[13]

Control 2,3-DPG values were obtained from eight nonpregnant female volunteers.

Results

Single observations. The results are summarized in Table I. A significant rise in 2,3-DPG values above the levels of nongravid control subjects was observed after the fifteenth week of gestation and was almost maintained through term and labor and post partum.

The data appeared nonlinear with a peak value at 19 to 22 weeks (Fig. 1) and, when examined by curvilinear correlation, were found to fit a quadratic function ($r = 0.19$; No. = 163; $P < 0.05$).

The hematocrit and corresponding 2,3-DPG values are also depicted in Fig. 1. An inverse relationship was suggested, but this impression was not statistically confirmed.

2,3-DPG levels were higher in the hypertensive patients when compared with normal gravid women at

the same stage of gestation. In view of the small numbers of hypertensive patients studied, significance was achieved only by grouping antepartum observations, those at 35 to 42 weeks, and the labor and postpartum observations (Table I). After this grouping, a significant difference in the antepartum period with the two-tailed t test was observed. The elevation of 2,3-DPG values in the labor–postpartum group was only of borderline significance ($p < 0.10$).

Maternal-newborn pairs.

Mothers. The results of the serial study are summarized in Table II. Significantly elevated levels of 2,3-DPG were again found in the hypertensive group during pregnancy (34 to 42 weeks). No significant difference in 2,3-DPG values between the hypertensive and normotensive gravid women could be demonstrated in labor or post partum.

Newborn infants. There was no significant difference in the mixed cord blood at delivery or in capillary blood on the third day of life in 2,3-DPG values between infants of hypertensive mothers and those born after normal pregnancies; however, only two of these infants of hypertensive mothers were small for their gestational age. In these cases, the 2,3-DPG values were 3.8 and 5.8 millimoles per milliliter, respectively.

Comment

Our finding of an increase in red blood cell 2,3-DPG which reached a peak value at 19 to 22 weeks of gestation is in partial disagreement with the results of Rorth and Bille Brahe,[3] who, in a limited study, reported a linear increase between the third and the ninth month of pregnancy. Unfortunately, in their study, no observations were made between the third and seventh months. The mechanisms responsible for the increase in 2,3-DPG are not completely understood. The role of 2,3-DPG in the delivery of oxygen to peripheral tissues is firmly established. Oxygen consumption in pregnancy increases with progressive gestation,[14] and total blood volume and red cell mass increase during pregnancy in order to meet this increased demand of oxygen transport. Plasma and red blood cell volume increase in different proportions during pregnancy, and, as a result, the mean whole-body hematocrit reaches its lowest point between 21 and 24 weeks.[15] An inverse relationship between total body red cell mass and 2,3-DPG levels has been described as a compensatory mechanism in anemic patients.[16] The inverse relationship between hematocrit and 2,3-DPG values observed in this study, although not statistically significant, appear to lend support to the concept of functional anemia which is most pronounced in midpregnancy.

The erythrocyte 2,3-DPG concentration may also be affected by maternal acid-base changes. A direct relationship between pH values and 2,3-DPG concentration has been reported.[17] Mild respiratory alkalosis with a slight but significant rise in pH has been described throughout pregnancy[18] and may further increase 2,3-DPG values. Values in labor and early post partum may be associated with the mild rise in pH values during labor.[19] Furthermore, muscular exercise has been associated with 2,3-DPG elevation.[20] Therefore, uterine work, which is similar to other muscular exercise, may explain the additional increase in 2,3-DPG levels during labor and early post partum.

Hypertension in pregnancy is known to be associated with generalized as well as uteroplacental vasoconstriction, resulting in diminished uteroplacental flow and intrauterine hypoxia.[8] A rise in 2,3-DPG is known to occur with a variety of hypoxic conditions not necessarily associated with red blood cell deficit.[4, 6, 21] Most authors interpret this increase as a compensatory mechanism to aid in oxygen release to peripheral tissues. Therefore, the 2,3-DPG elevation observed in the hypertensive group during pregnancy may be interpreted as a further compensatory mechanism to improve tissue and placental oxygen transfer beyond that observed in normal pregnancy.

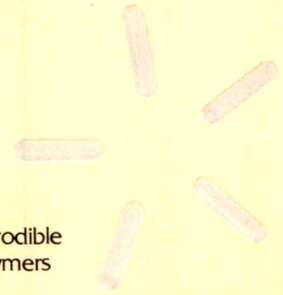
2,3-DPG values in the newborn infants of hypertensive mothers were not significantly different from those of control newborn infants. Whereas 2,3-DPG binding to fetal cells seems to affect oxygen release only to a minor extent,[22] a decrease in 2,3-DPG in intrauterine malnourished infants may be of importance.[11] This difference was not apparent in our study as only two of our infants were truly small for their gestational ages.

An increase in 2,3-DPG will shift the maternal dissociation curve to the right and facilitate oxygen release, including placental transfer. If 2,3-DPG in the fetus with intrauterine growth retardation declines, then the fetal dissociation curve would have a slight left shift. This would further increase the maternal-fetal oxygen pressure gradient at the placental site and facilitate oxygen transfer to the fetus. Further research in this area may help to clarify the compensatory role of fetal and maternal 2,3-DPG changes in "distressed" pregnancies.

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Bioerodible
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Oral Osmotic
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Alza is the only company devoted entirely to the optimization of drug therapy through the development of Therapeutic Systems. These "Systems" are vehicles for precision drug delivery with minimal physiologic disturbances and minimal reliance on patient compliance.

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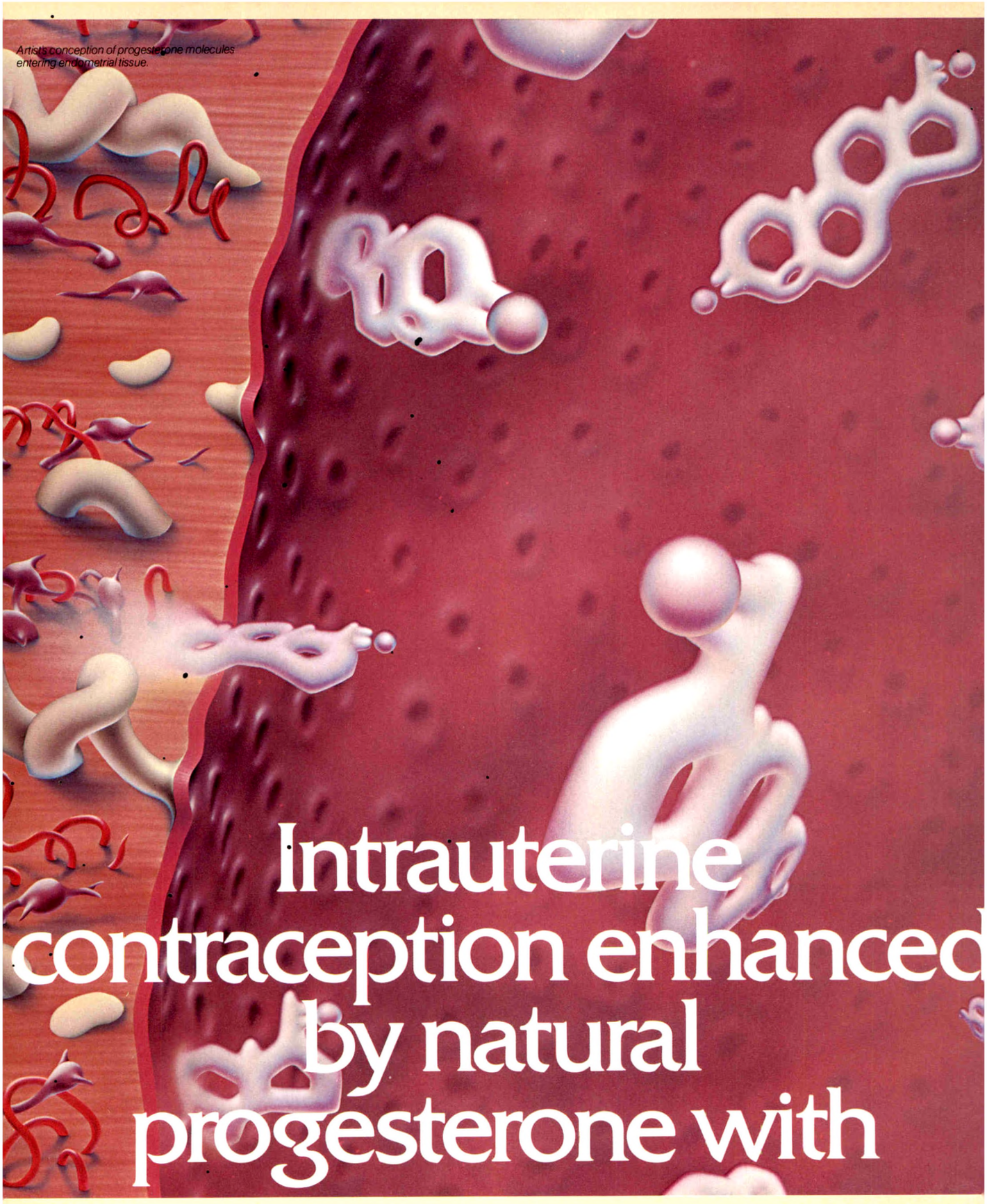


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Alza is different from the ordinary pharmaceutical company in that it is not involved in manipulating or developing the architecture of existing drug molecules. Alza is the Therapeutic Systems Company.



Liquid Infusion
System



Artist's conception of progesterone molecules entering endometrial tissue.

Intrauterine contraception enhanced by natural progesterone with

no systemic hormonal effects*

e product information section for possible IUD side effects.

The Progestasert® System is a new approach to intrauterine contraception.

The natural ovarian hormone progesterone is placed to act continuously directly within the target organ—the uterus. Thus, only minute quantities of this rapidly catabolized hormone are needed.

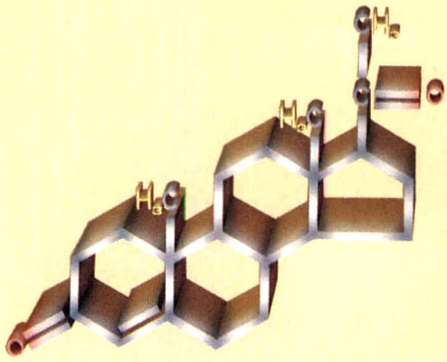
Hormonally enhanced contraception is achieved in utero without systemic side effects* and without affecting the hypothalamic-pituitary-ovarian axis. Effective contraception is achieved for a full year from a single Progestasert® System placement.

Alza introduces Progestasert®

Intrauterine Progesterone Contraceptive System

*See product information section for possible IUD side effects.
See last page for prescribing information.

Contraceptive efficacy of the Progestasert® System enhanced by progesterone



WHY PROGESTERONE?

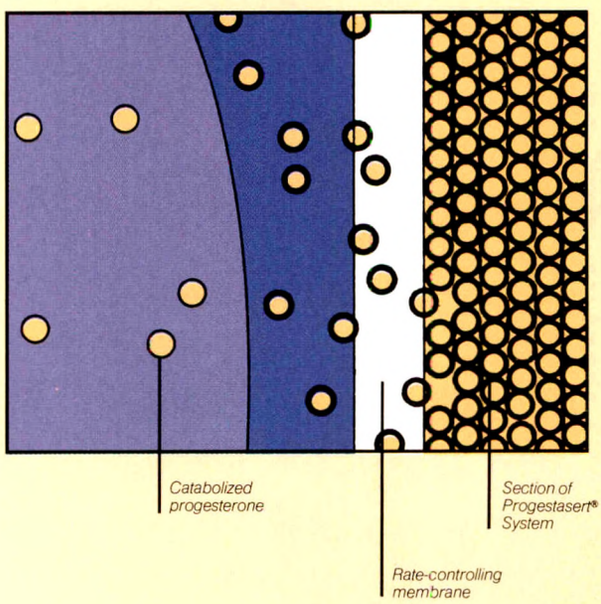
Progesterone— pregn-4-ene-3,20-dione ($C_{21}H_{30}O_2$)— is a naturally occurring female hormone, produced by the corpus luteum, adrenal cortex and placenta.

Progesterone is the contraceptive agent of choice for the Progestasert® System because:

- ☐ it is endogenous to the endometrium
- ☐ it has a short metabolic half-life
- ☐ it enhances the contraceptive effect at low concentrations when administered locally to the uterus
- ☐ it is rapidly catabolized by the endometrium
- ☐ it permits continuation of ovulation and, therefore, the length of the menstrual cycle is unaltered
- ☐ its effect on the endometrium reverses rapidly upon removal of the Progestasert® System.

CONTINUOUS MICRODOSAGE OF PROGESTERONE

The total quantity of progesterone contained in one Progestasert® System for one year's use is only 38 mg— less than the amount produced in one day by the corpus luteum during the latter part of the menstrual cycle. The amount of progesterone released daily from the System corresponds to a small percentage of the daily physiologic production.



A unique drug delivery system

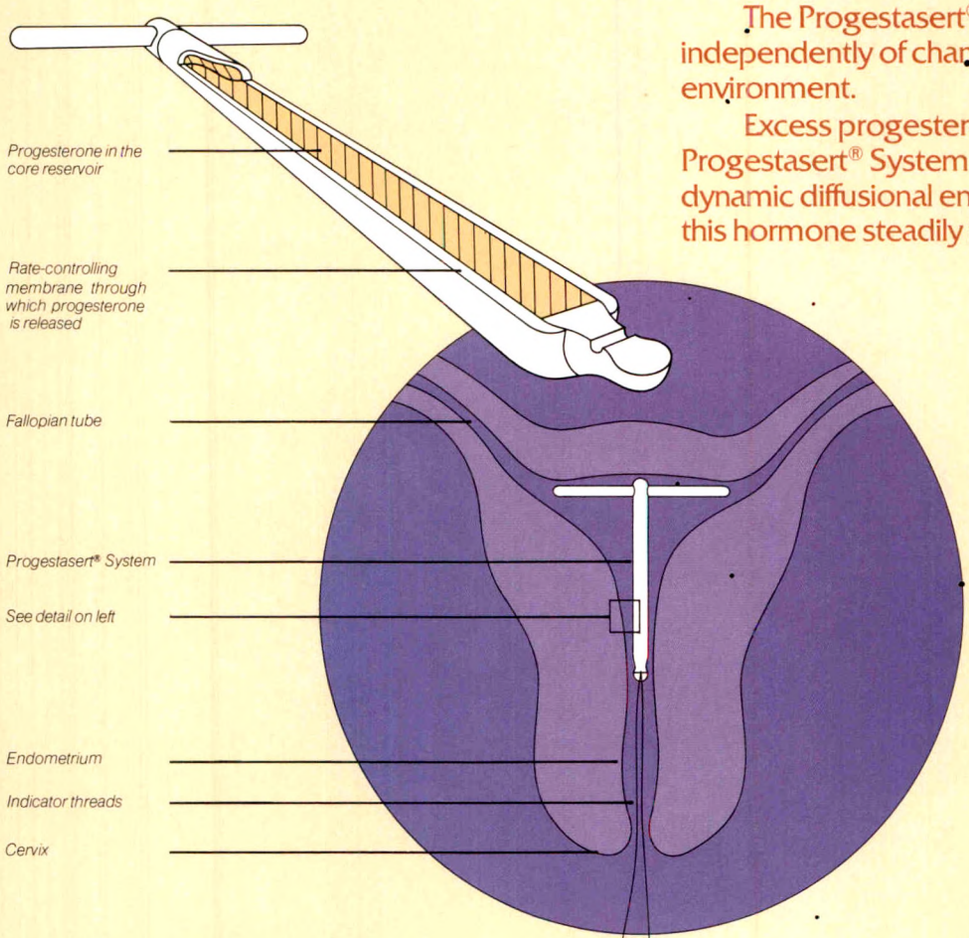
The Progestasert® System is a diffusional system designed to deliver microquantities of progesterone. The two key components are:

- ☐ a core reservoir of progesterone crystals with barium sulfate (for radiopacity) in medical-grade silicone oil
- ☐ an inert diffusional membrane of ethylene/vinyl acetate copolymer that surrounds the core reservoir and provides programmed control of progesterone release

When the Progestasert® System is placed in the uterine environment, progesterone molecules diffuse through the membrane into the uterine lumen at the average rate of 65 micrograms per day for one year.

The Progestasert® System functions independently of changes in the uterine environment.

Excess progesterone within the Progestasert® System provides the thermodynamic diffusional energy needed to release this hormone steadily for an entire year.



WHY THE T CONFIGURATION?

A T-shaped configuration was selected to contain the progesterone reservoir because this configuration has shown:*

- ☐ easy insertion
- ☐ excellent retention by the uterus
- ☐ minimal symptomatic evidence of uterine irritation
- ☐ easy removal

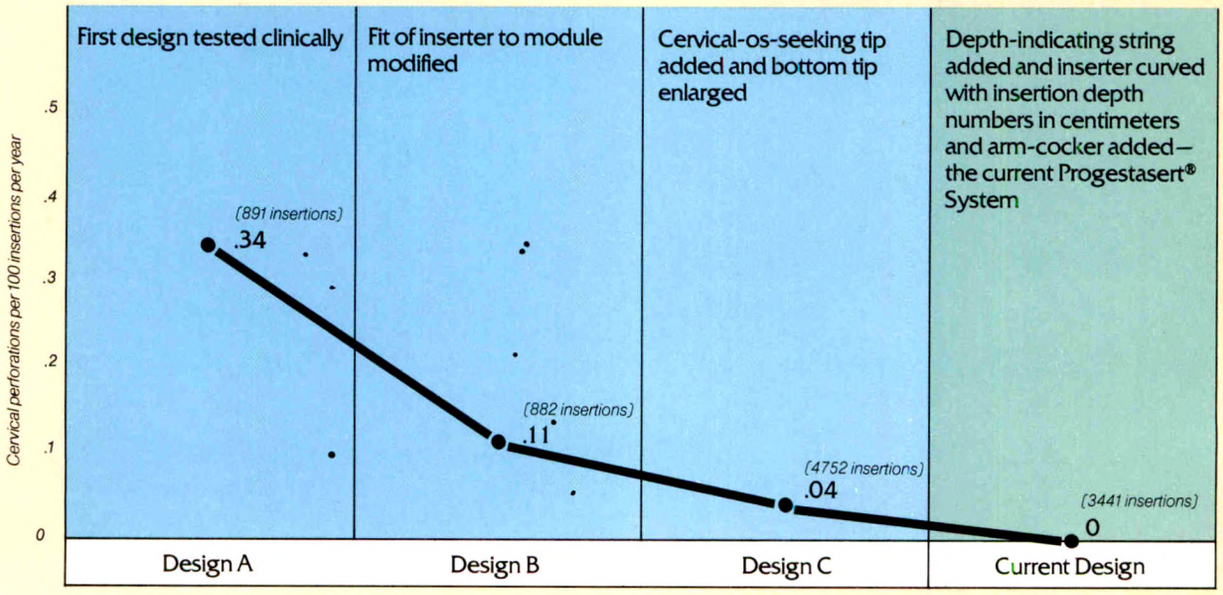
*Tatum HJ: Intrauterine Contraception. American Journal of Obstetrics and Gynecology, 112: 1000, 1972.

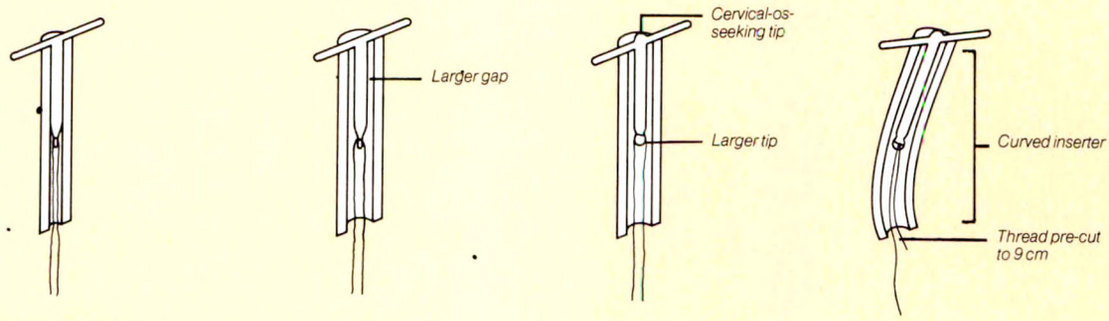
Progestasert®

Intrauterine Progesterone Contraceptive System

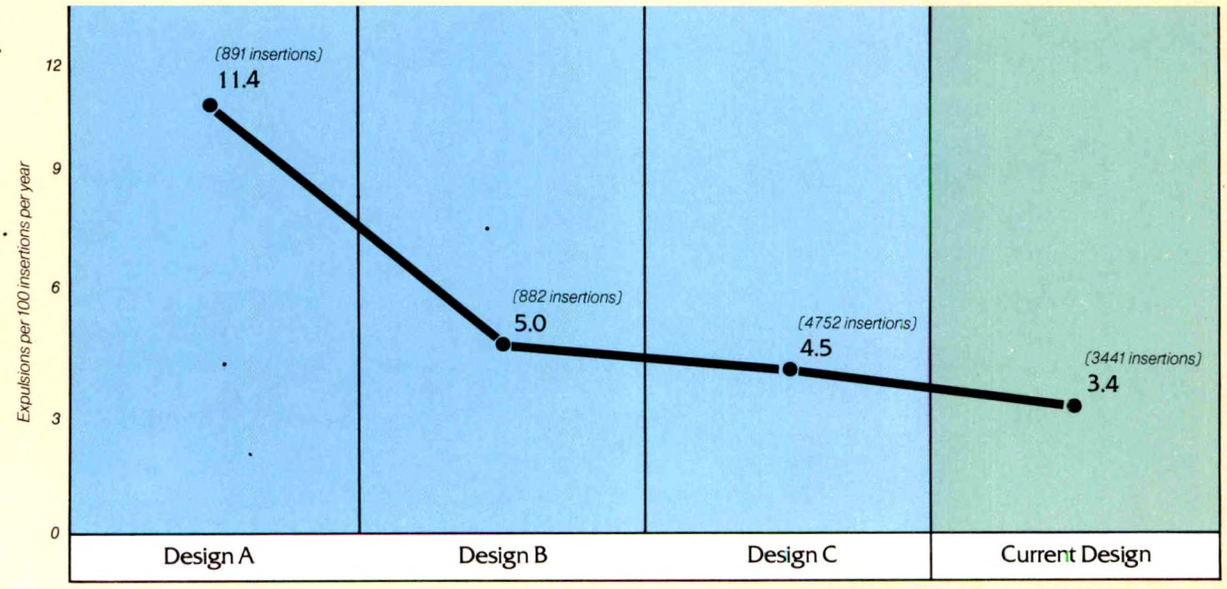
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CERVICAL PERFORATIONS REDUCED AS ALZA'S SYSTEM AND INSERTER DESIGN IMPROVED





EXPULSIONS REDUCED AS ALZA'S SYSTEM AND INSERTER DESIGN IMPROVED



These graphs do not represent a comparison of inserters produced by different manufacturers. Rather, they depict stages in ALZA's development of the Progestasert® System inserter.

All numbers are expressed as events per 100 women per year.

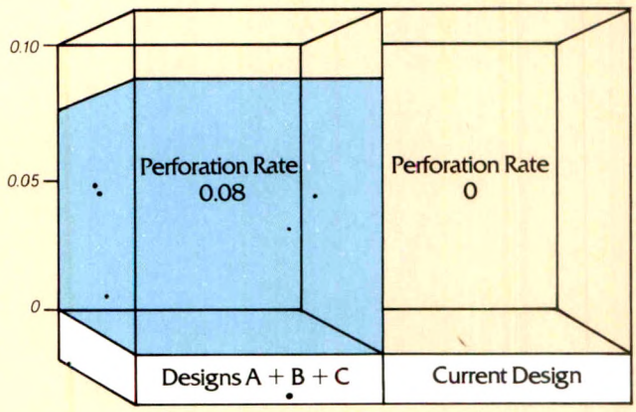
Inserter designed for safety and effective placement

As recently noted by Mishell, inserter design is increasingly recognized as important to the efficacy and safety of intrauterine contraceptive devices. "Proper placement…is of extreme importance for IUD effectiveness… (Thus), more attention should be directed toward the development of optimal IUD inserters than to changes in the IUD design."*

Some untoward effects with intrauterine devices— such as perforation and expulsion— in the past have been due to difficulties in placement. Since the technique of insertion and correct high fundal positioning play an important role in assuring contraceptive effectiveness and patient comfort and safety, much attention has been directed toward the development of the Progestasert® System inserter.

In controlled studies, evolutionary changes in the inserter and the Progestasert® System have demonstrated a decrease in the incidence of expulsions, cervical perforations, and uterine perforations which have paralleled these design changes. The incidence for both cervical and uterine perforations has decreased to the point that neither type of perforation has occurred with the 3,441 insertions with the final design after an average of more than nine months of exposure for these women.

UTERINE PERFORATIONS REDUCED AS ALZA'S SYSTEM AND INSERTER DESIGN IMPROVED



While these data reflect the importance of the design changes, the lack of perforations during these limited trials in no way suggests the Progestasert® System and its inserter will be trouble-free in general use. Perforations, both cervical and uterine, may occur with the Progestasert® System as its use becomes more widespread. The rate of perforation will depend upon anatomical and physiological variations of the women receiving the System and the care taken during the insertion process.

Progestasert®

Intrauterine Progesterone Contraceptive System

*Mishell DR, Jr: Assessing the Intrauterine Device. Family Planning Perspectives 7:103, 1975

See last page for prescribing information.

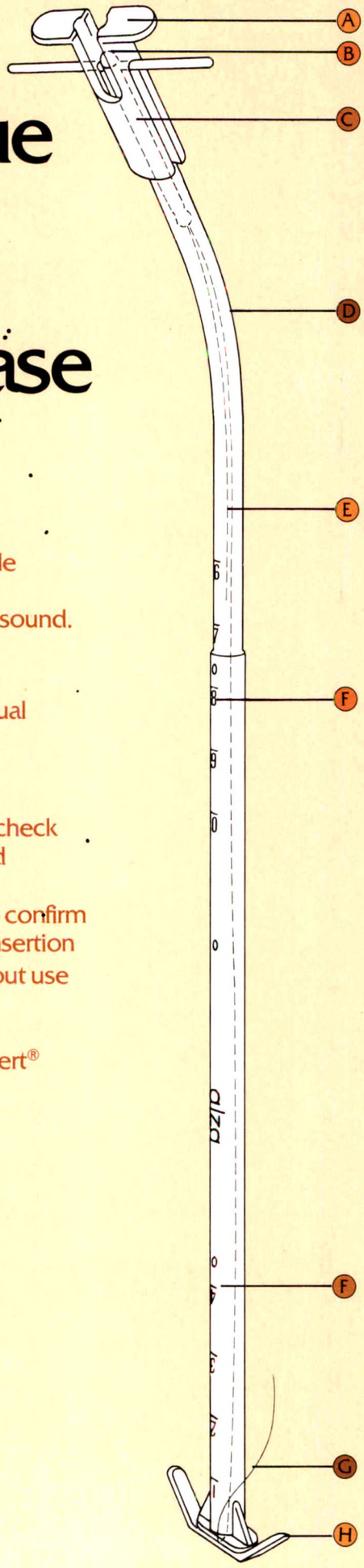
The System and its unique inserter-designed for safety and ease of insertion

The Progestasert® System inserter facilitates correct System placement while minimizing the risk of perforation, and is designed to function much like a uterine sound.

Features of the inserter:

- ☐ pre-curvature to facilitate insertion
- ☐ malleability for adapting to the individual uterus
- ☐ dual checking mechanism for correct placement
  - — centimeter markings on inserter to check conformity with previously sounded uterine depth
  - — indicator thread length designed to confirm proper System position following insertion
- ☐ high fundal placement achieved without use of plunger or push-rod

Comprehensive illustrated insertion instructions are a part of every Progestasert® System package.



Actual Size



A. Arm-cocker allows the arms of the T to be folded aseptically, using one-handed technique just prior to insertion.

B. Cervical-os-seeking tip helps guide the System into the cervical canal, making insertion easier and more comfortable.

C. Vertical stem of the System designed with flexibility and rounded tip to minimize cervical perforation risk.

D. Curved portion of inserter designed to conform to anatomic configuration of most cervical-uterine cavities. Malleability allows accomodation to variations in uterine anatomy.

E. Indicator monofilament thread, extended 9 cm from the top of the System, verifies correct placement at insertion.

F. Graduations (in centimeters) to determine uterine position and depth, and to confirm correct System placement.

G. Retainer monofilament thread extends the length of the inserter and is anchored by the thread-retaining plug to retain the System in the inserter.

H. Thread-retaining plug is removed to release the System in the uterus.

Safety studies

Clinical safety studies demonstrated:

- No extrauterine metabolic or endocrine effects in humans*
- Endometrial changes induced by the Progestasert® System disappear within weeks after System removal
- Rapid return to fertility confirmed in women discontinuing use of the Progestasert® System in the hope of becoming pregnant. The cumulative conception rate among 154 women was 49 per 100 women pregnant at 3 months after removal, and 76 per 100 women at 12 months after removal. These rates of return to fertility are similar to those reported following removal of an IUD.

Effective contraception for one year from a single placement

The Progestasert® System functions unattended for one year at a continuous release rate of 65 micrograms of progesterone per day, eliminating reliance on daily hormone administration.

Yearly removal and placement can coincide with annual visits for physical examination and Pap smear, as encouraged by the American College of Obstetrics and Gynecology. (Currently accepted practice used by recognized authorities, reported in ACOG Technical Bulletin #29, February, 1975, p 7.)

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Progestasert®

Intrauterine Progesterone Contraceptive System

*See product information section for possible IUD side effects.
See last page for prescribing information.

Some clinical aspects

Data were collected in 40 clinics involving 5,495 women over a period of 45,848 woman-months. These trials were designed to obtain data that would predict the true use-effectiveness of the System.

HIGHLY EFFECTIVE INTRAUTERINE CONTRACEPTION

Clinical results have demonstrated pregnancy rates of 1.9† in parous patients and 2.5† in nulliparous patients. A similar T-shaped unit, but without progesterone in the stem, produced a pregnancy rate of 18.3†.

HIGH DEGREE OF ACCEPTABILITY

Clinical studies have demonstrated a high continuation rate. Approximately 8 of every 10 parous and 7 of every 10 nulliparous women continued use of the Progestasert® System for a full year.

SHAPE PROVIDES EXCELLENT RETENTION

Expulsion rates of 3.1† and 7.5† were observed for parous and nulliparous women respectively.

INSERTER PROVIDES SAFE PLACEMENT

No uterine perforations have occurred with 3,441 insertions using the current pre-curved, malleable inserter during controlled clinical trials. This should not be interpreted as a guarantee that perforations will not occur with the Progestasert® System.

	Parous	Nulliparous
PREGNANCY RATE	1.9	2.5
EXPULSIONS	3.1	7.5
CONTINUATION RATE	79.1	70.9

†*All numbers are expressed as events per 100 women per year.*

Features and benefits

- ☐ Contraception provided by progesterone, identical to the natural ovarian hormone
- ☐ No known systemic hormonal effects*
- ☐ Continuous delivery of progesterone in microgram amounts/day
- ☐ Effective contraception for one year from one System placement
- ☐ High degree of patient acceptability
- ☐ Excellent uterine retention
- ☐ Minimal reliance on patient compliance during its year of use when compared to extrauterine contraceptives
- ☐ Prompt return to pre-insertion fertility following System removal
- ☐ Inserter designed for safety and ease of insertion

*See product information section for possible IUD side effects

Candidates for the Progestasert® System

THE NEW CONTRACEPTIVE USER

The natural progesterone contraception provided by the Progestasert® System without systemic hormonal effects, along with minimal dependence on patient compliance, small unit size, and easy insertion, make the Progestasert® System an attractive first contraceptive for many women.

THE NONCOMPLIER

The daily regimen of pill-taking proves beyond the capability of some patients for whom hormonal contraception is preferable. The Progestasert® System reduces requirements for patient compliance to once-a-year replacement.

THE PATIENT DISCONTINUING ORAL CONTRACEPTIVES

Patient-initiated—the Progestasert® System offers the opportunity to continue hormonally enhanced contraception without the adverse systemic hormonal effects* that cause many women to dicontinue oral contraceptives.

Physician-initiated—some physicians consider it important for the patient to discontinue oral contraceptive use until the body regains its normal hormonal balance. Some physicians discontinue the Pill permanently. Recent studies strongly suggest that oral contraceptive users are at greater risk of developing coronary thrombosis than nonusers. The Progestasert® System, with its lack of adverse systemic hormonal effects,* can be the desired alternative contraceptive for former users of the Pill.

THE PATIENT DISCONTINUING OTHER CONTRACEPTIVE METHODS

The inconvenience and lower efficacy rates of diaphragms and condoms make the Progestasert® System an attractive alternative for the patient desiring intrauterine hormonal contraception without systemic hormonal effects.*

THE IUD PATIENT DUE FOR REINSERTION

For those patients already using some form of intrauterine contraception, the Progestasert® System, designed for safety, offers an effective alternative to their present IUD and may be considered at reinsertion time.

Progestasert®

Intrauterine Progesterone Contraceptive System

*See product information section for possible IUD side effects.
See last page for prescribing information.

Ordering Information

IF YOU WOULD LIKE FURTHER INFORMATION ABOUT ALZA, THERAPEUTIC SYSTEMS, THE PROGESTASERT® SYSTEM, DETAILED INSERTION INSTRUCTIONS OR WOULD LIKE TO ORDER PROGESTASERT® SYSTEMS FOR YOUR PRACTICE, CALL THE FOLLOWING TOLL-FREE NUMBER: (800) 227-9953 OR WRITE TO ALZA PHARMACEUTICALS, 3170 PORTER DRIVE, PALO ALTO, CALIFORNIA 94304.

Progestasert®

Intrauterine Progesterone Contraceptive System

Release rated 65 μg/day progesterone for one year.

DESCRIPTION

The Progestasert® Intrauterine Progesterone Contraceptive System is a white, T-shaped unit constructed of ethylene/vinyl acetate copolymer (EVA) containing titanium dioxide. The 36 mm tubular vertical stem of the T contains a reservoir of 38 mg of progesterone, USP, together with barium sulfate, USP, for radiopacity; both are dispersed in medical grade silicone oil. The 32 mm horizontal crossarms are solid EVA. Two monofilament nylon indicator/retrieval threads are fastened to the base of the T stem. The Progestasert® system is packaged sterile within its curved, malleable inserter. Progesterone is released from the system at an average rate of 65 μg/day for one year by membrane-controlled diffusion from the reservoir. None of the inert ingredients of the reservoir or membrane—barium sulfate, silicone oil, titanium dioxide, or EVA—is released from the system.

ACTION

Available data indicate that the contraceptive effectiveness of the Progestasert® system is enhanced by its continuous release of progesterone at an average rate of 65 μg/day into the uterine cavity. The mechanism by which progesterone enhances the contraceptive effectiveness of the T is local, not systemic. The concentration of luteinizing hormone, estradiol, and progesterone in systemic venous plasma follow regular cyclic patterns indicative of ovulation during use of the Progestasert® system. Blood chemistry studies related to liver, kidney, and thyroid function also reveal no changes.

During use of the system, the endometrium shows progestational influence. Following removal of the system, the endometrium rapidly returns to its normal cyclic pattern and can support pregnancy. The local mechanism by which continuously released progesterone enhances the contraceptive effectiveness of the T has not been conclusively demonstrated. The hypotheses that have been offered are: progesterone-induced inhibition of sperm capacitation or survival, and alteration of the uterine milieu so as to prevent nidation.

INDICATION

The Progestasert® system is indicated for contraception.

CONTRAINDICATIONS

Pregnancy or suspicion of pregnancy; abnormalities of the uterine cavity; acute pelvic inflammatory disease or history of repeated pelvic inflammatory disease; postpartum endometritis or infected abortion in the past 3 months; uterine or cervical malignancy; unexplained genital bleeding until suspicion of cancer is ruled out; acute cervicitis.

WARNINGS

A. Pregnancy

1. Septic abortion. Recent reports have indicated an increased incidence of septic abortion, associated in some instances with septicemia, septic shock and death in patients becoming pregnant with an intrauterine device (IUD) in place. In some cases, the initial symptoms have been insidious and not easily recognized. If pregnancy should occur with a Progestasert® system in situ, it should be removed if the thread is visible or, if removal proves to be or would be difficult, interruption of the pregnancy should be considered and offered as an option. If the patient elects to maintain the pregnancy and the system remains in situ, she shall be warned of the increased risk of sepsis and be followed with close vigilance.
2. Congenital anomalies. Systemically administered sex steroids, including synthetic progestational agents, have been associated with an increased risk of congenital anomalies including cardiovascular anomalies. Such effects have not been reported with progesterone itself, which is produced in large concentrations during pregnancy.
3. Ectopic pregnancy. A pregnancy that occurs while a patient is wearing an IUD is much more likely to be ectopic in nature. Therefore, such patients in whom pregnancy occurs should be carefully evaluated.

B. Severe Pelvic Infection

Occasionally serious pelvic infection may occur with an IUD in situ. Under such circumstances the IUD should be removed, appropriate bacteriological studies done, and appropriate antibiotic treatment instituted.

C. Perforation

Perforation of the uterus may occur. If this occurs, the device should be removed. Local inflammatory reaction with abscess formation is a possibility if an IUD is left in the abdomen. Cervical perforation, often asymptomatic, has also been reported. If such occurs, the device should be removed.

PRECAUTIONS AND ROUTINE EXAMINATIONS

A. Patient Examination

The physician should make a determination that the patient is not pregnant. The possibility of insertion in the presence of an existing undetermined pregnancy is reduced if insertion is performed during or shortly following a menstrual period. The system should not be inserted postpartum or postabortion until involution of the uterus has been completed. Prior to insertion, a pelvic examination, pap smear, and gonorrhea culture should be done. Sound the uterus prior to insertion and exercise care to avoid perforation. Do not use excessive force. The patient should be told that some bleeding or cramps may occur during the first few days after insertion. If these symptoms continue or are severe, she should report to her physician. She should be cautioned not to pull on the threads, which would displace the system.

B. Requirements for Continuation and Removal

The Progestasert® system must be replaced each year. There is no evidence of decreasing contraceptive efficacy with time before one year, but the contraceptive effectiveness of the Progestasert® system after one year has yet to be established. Therefore, the patient should be informed of the known duration of the contraceptive efficacy and be advised to return in one year for re-examination, removal and possible reinsertion.

The Progestasert® system should be removed for the following medical reasons: Menorrhagia and/or metrorrhagia producing anemia; uncontrolled pelvic infection; intractable pain, often aggravated by intercourse; pregnancy, if the thread is visible; uterine or cervical malignancy; uterine or cervical perforation; an increase in length of thread extending from the cervix, or any other indication of partial system expulsion.

C. Continuing Care of Patients Using the Progestasert® System

Patients should be re-examined and evaluated within three months after Progestasert® system insertion. Routine annual examination, removal of the Progestasert® system, and reinsertion of a new Progestasert® system along with appropriate medical and laboratory evaluation should be carried out.

ADVERSE REACTIONS

Reported adverse reactions include: Endometritis, spontaneous abortion, septic abortion, septicemia, perforation of uterus and cervix, pelvic infection, cystic masses in pelvis, abdominal adhesions, intestinal obstruction, cervical erosion, vaginitis, leukorrhea, cystitis, pregnancy, ectopic pregnancy, uterine embedment, difficult removal, complete or partial expulsion, intermenstrual spotting, prolongation of menstrual flow, anemia, amenorrhea or delayed menses, pain and cramping, backaches, dyspareunia, leg pain or soreness, weight loss or gain, nervousness, bradycardia and syncope secondary to insertional pain.

DOSAGE AND ADMINISTRATION

A single Progestasert® system is to be inserted into the uterine cavity (see Precautions section). Present information indicates that the contraceptive effectiveness of the system is retained for one year and the system must be replaced one year after insertion.

HOW SUPPLIED

Available in cartons containing six sterile systems (Progestasert® system with an inserter).

CLINICAL STUDIES

Different event rates have been recorded with the use of different contraceptive methods. Inasmuch as these results are usually derived from separate studies conducted by different investigators in several population groups, they cannot be compared with precision. Furthermore, event rates tend to be lower as clinical experience is expanded, possibly due to retention in the clinical study of those patients who accept the treatment regimen and do not discontinue due to adverse reactions or pregnancy. In clinical trials sponsored by Alza with the Progestasert® system, use-effectiveness was determined for parous and nulliparous women by the life table method. (Rates are expressed as events per 100 women through 12 months of use.) This experience is based on 45,848 woman-months of use with 5,495 women, of whom about 25% were nulliparous; 2,157 women completed 12 months of use.

	Parous	*Nulliparous*
Pregnancy	1.9	2.5
Expulsion	3.1	7.5
Medical Removals	12.3	16.4
Continuation Rate	79.1	70.9

CAUTION

Federal law prohibits dispensing without prescription.

alza pharmaceuticals

FETUS, PLACENTA, AND NEWBORN

Strangulation of umbilical cord by amniotic band

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Congenital malformation caused by amniotic bands is an extremely rare entity. A case report of a fetal death caused by constriction of the umbilical cord by an amniotic band which joined both hands is presented.

THE MOST COMMON congenital malformations caused by amniotic bands involve the extremities. This type of malformation occurs about once in every 10,000 live births and presents most often as distal syndactyly.[1] When twisted around the umbilical cord, the amniotic band may cause fetal death, but this event is extremely rare, with only 12 cases noted in a review of the world literature up to 1959. We recently observed an amniotic band joining both hands, with malformation of one hand and also constriction of the umbilical cord, resulting in fetal death. This unusual configuration of a single amniotic band has not previously been described.

Case report

A. G., a 26-year-old oriental woman, para 2, gravida 3, was hospitalized because of vaginal bleeding in the twenty-second week of gestation. The pregnancy was previously unremarkable. The uterus was soft, and a gestational age of only six weeks was estimated. The cervix was dilated, with placental tissue presenting at the os. The uterus was evacuated on the day of admission by use of polyp and ring forceps, followed by suction.

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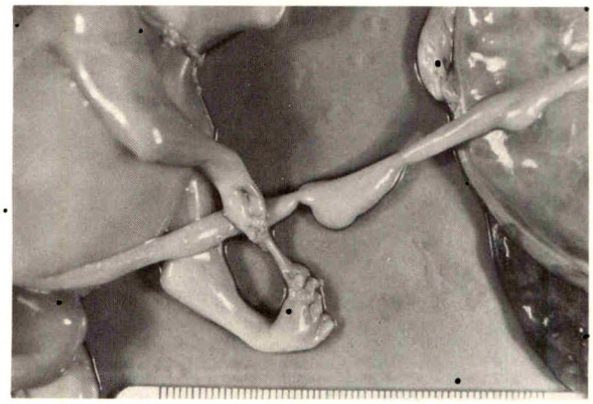


Fig. 1. Amniotic band joining hands near constriction of umbilical cord.

The intact amniotic sac contained a slightly macerated male fetus with a crown-to-rump measurement of 9.5 cm. There was a strong fibrous-appearing band joining a deformed right hand with the fourth and fifth fingers of the left hand and also tightly constricting the adjacent umbilical cord (Fig. 1.). The right thumb was partly fused to the hand, and the remaining four fingers were joined and rudimentary. The band was 1 cm. long and 0.1 cm. in diameter at its midpoint and required considerable force for removal from the umbilical cord constriction. Histologic study of the band showed chorionic connective tissue with a covering of somewhat degenerate amniotic epithelium.

The etiology of the amniotic band is uncertain. Torpin[2] suggested that the amnion may rupture or

separate from the chorion, with subsequent development of sheets or bands that may encircle fetal parts to produce constriction rings or amputation or may wrap around the neck or umbilical cord. Amniotic bands in all previous cases of strangulation of the umbilical cord were either still attached to the placenta at delivery or detached without congenital fetal malformations, and all were cases at or near term. The very strong attachment of the amniotic band with malformation of the hand in our case suggests that the attachment to the hands occurred before the constriction of the umbilical cord.

If the amniotic band is caused by early rupture of the amnion, this may possibly be a hazard of amniocentesis in early pregnancy. Baker and Rudolph[1] suggested that pediatricians should be aware of a possibly increasing incidence of congenital malformations caused by amniotic bands with the increasing use of amniocentesis.

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The effect of methadone on fetal brain function

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MAIDA LIU, B.S.
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The effect of methadone on fetal brain function in terms of the elctroencephalogram (EEG) was studied in eight short-term fetal sheep experiments by single injections of 5 to 20 mg. of methadone into the fetal circulation. Plasma methadone concentrations (determined by radioimmunoassay) greater than 0.065 mg. per cent were associated with an immediate but brief decrease in carotid blood flow, a rapid decrease in frequency and amplitude in the EEG that occasionally became isoelectric but recovered, and immediate and relatively prolonged bradycardia. Similar cardiovascular observations were obtained with plasma methadone concentrations less than 0.065 mg. per cent, but the EEG changes were either minimal or not observed. These results indicate that uptake of methadone by the fetal brain is rapid and that electrical activity and heart rate are affected.

SEVERAL REPORTS have appeared in the literature in recent years that report the effect of methadone on pregnancy and the newborn infant. The increased interest in this topic has arisen because of the successful experience with methadone in detoxification and maintenance programs for heroin addicts. The reports to date generally indicate that with comprehensive prenatal care methadone-treated women appear to have no greater obstetric risk than a nonaddicted group of women with similar demographic and obstetric history characteristics.[1] However, as many as 50 per cent of methadone-treated women do not receive prenatal care and a higher incidence of obstetric complications, particularly prematurity and intrauterine growth retardation, is reported in these women.[2, 3] All investigators have reported withdrawal symptoms in the newborn infants requiring pharmacologic treatment in approximately 30 per cent of cases. Late presentation of drug withdrawal symptoms of infants two to three weeks of age whose mothers had received methadone has been reported.[4] The high incidence of withdrawal symptoms in the newborn infant indicate clearly that methadone passes the placental barrier and accumulates within the fetus and amniotic fluid.[5] The uptake of methadone by the brain has been shown to be very rapid.[9] In vivo physiologic observations of the effect of methadone on the fetus have not been reported. It is the purpose of this report to present the observations of experiments conducted in fetal sheep to study the effect of methadone on the fetus.

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Material and methods

Experiments were performed on eight Dorset ewes with dated gestations that ranged from 119 to 139 days (term, approximately 145 days). The ewes were prepared for operation by restricting food for 24 to 48 hours and water for 12 hours. The ewes received progesterone in oil,* 150 mg. twice daily, for two days before and on the day of operation. Anesthesia was induced with halothane,† and maintained at a concen-

*Proluton, Schering Corp., Union, New Jersey.

†Fluothane, Ayerst Labs., Div. American Home Products Corp., 685 Third Ave., New York, New York 10017.

tration of 0.6 to 1.2 per cent with 50 per cent oxygen delivered through a cuffed endotracheal tube by means of an anesthetic apparatus and negative-positive-pressure respirator.*

The fetal head was delivered through a small incision in an avascular area of myometrium and placed into a stereotaxic device† that was modified for fetal sheep by methods described previously.[7] The technique of catheterization of the carotid artery and sagittal vein has been presented previously in detail.[8] A perivascular ultrasonic flow transducer‡ was placed high on the carotid artery after tributaries had been ligated. In vivo calibration of the flow transducer was performed as described previously.[8] Pressure in the cerebral vessels was monitored continuously via pressure transducers.§ Heart rate was calculated from the pressure tracings.

Continuous bipolar recording of the electroencephalogram (EEG) was obtained from extradurally placed solder ball electrodes by the technique described previously.[9] All electrophysiologic parameters were displayed by direct write-out on an eight-channel dynagraph‖ through appropriate couplers.

A second small incision was made in the myometrium over the hind limbs, then either a catheter was passed into an umbilical vein via a tributary, or a hind limb was delivered and a catheter was passed into the fetal inferior vena cava via the femoral vein.

Blood samples were drawn from the fetal vessels prior to and at frequent intervals following the injection of a 5 to 20 mg. bolus of methadone hydrochloride (0.25 to 1 ml.)¶ into the umbilical vein, inferior vena cava, or sagittal vein. Part of the blood sample was immediately deproteinated with cold perchloric acid, and pH, P_{CO_2}, and P_{O_2} were determined immediately with a pH blood gas system# on part. Plasma methadone levels were determined with a recently developed radioimmunoassay method** by the Toxicology Division, Office of the Medical Examiner, Nassau County.[10] Glucose determinations were performed on the deproteinated sample by enzymatic methods, the materials for which are supplied in convenient kits.*

Statistical analysis of the data was performed by paired serial analysis from the base line through the recovery period following methadone injection.

Results

The initial experimental protocol involved maternal injections of methadone with simultaneous samplings of both maternal and fetal circulations in order to obtain an understanding of placental transport of methadone as well as its effect on the fetus. Maternal injection of methadone was discontinued after relatively large dosages (50 to 150 mg.) given to the ewe failed to reveal levels of methadone in the fetal circulation that were greater than the sensitivity of the assay (0.01 mg. per cent). Therefore, a smaller bolus of methadone was injected directly into the fetal circulation. Following the injection of methadone, fetal blood methadone levels increased rapidly, and, by 15 seconds to 4 minutes, levels of 0.07 to 0.09 mg. per 100 ml. of plasma were obtained. Thirty to forty-five minutes after injection, plasma methadone levels were 30 to 50 per cent of the peak concentration. No evidence of fetal-to-maternal transport of methadone was detected.

Fetal metabolic observations. Following methadone injection, there was no significant difference from base line noted for pH (base line 7.359 ± 0.026 standard error of the mean), oxygen tension (20.3 ± 1.2 mm. Hg), carbon dioxide tension (42.0 ± 2.1 mm. Hg), and glucose (17.1 ± 1.7 mg. per cent). The cerebral metabolic rate for oxygen, glucose, and carbon dioxide was not altered significantly following the methadone injection. The cerebral glucose/oxygen utilization ratio was not altered significantly following methadone (1.07 ± 0.06).

Fetal cardiovascular observations. Fetal heart rate, mean carotid-arterial pressure, and carotid blood flow were altered consistently and differed significantly from base line. The results of a single experiment are shown in Fig. 1. Almost immediately following the injection of methadone, a significant decrease in mean cerebral perfusion pressure and an increase in cerebral resistance were noted. As a result of these changes, blood flow in the carotid artery was depressed by as much as 50 per cent. These changes were short-lived, and, by 3 to 7 minutes after infusion, base line values were present in all experiments. Immediate fetal

*Bird, Palm Springs, California.

†David Kopf Instruments, 734 Elmo St., Tujunga, California 91042.

‡Ward Associates, LaJolla, California.

§P-23db, Statham Instruments, Inc., 2230 Statham Blvd., Oxnard, California 93030.

‖Type RM, Beckman Instruments, Inc., 2500 Harbor Blvd., Fullerton, California 92634.

¶Dolophine hydrochloride, Eli Lilly & Co., Box 618, Indianapolis, Indiana 46206.

#No. 113, Instrumentation Laboratory, Inc., 113 Hartwell Ave., Lexington, Massachusetts 02173.

**Abuscreen, Roche Diagnostics, Division of Hoffmann-La Roche Inc., Roche Park, Nutley, New Jersey 07110.

*Boehringer Mannheim Corp., 219 E. 44th St., New York, New York 10017.

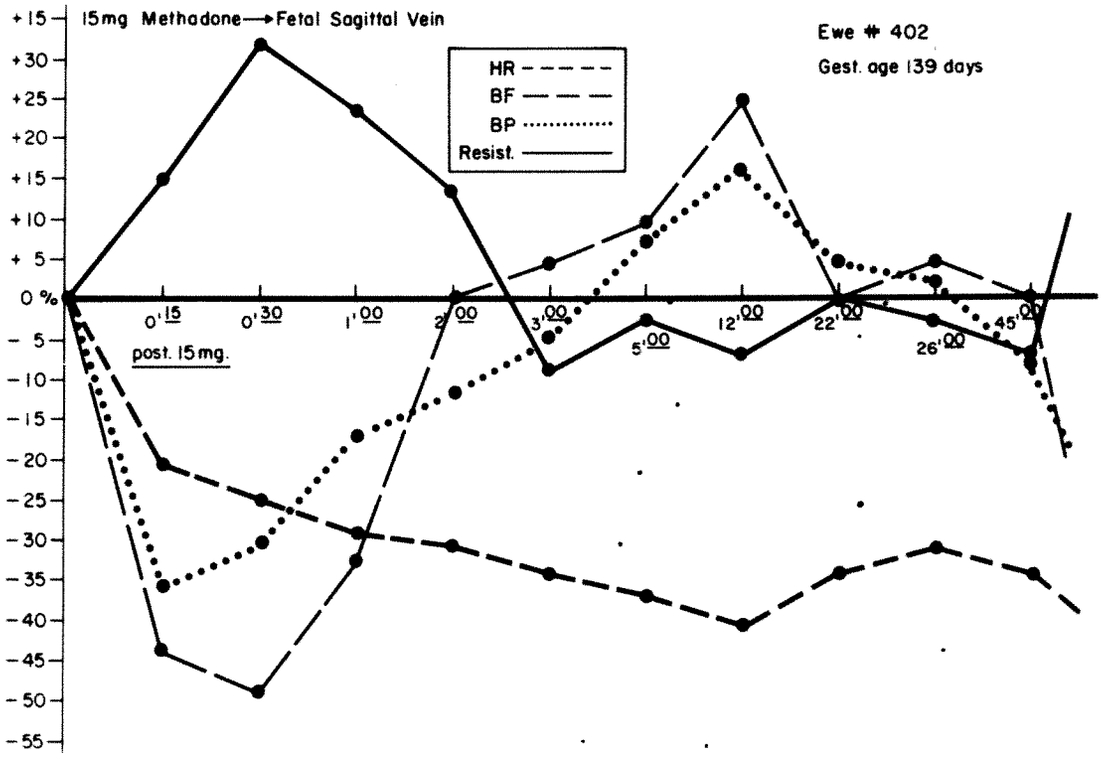


Fig. 1. Fetal heart rate (*HR*), carotid blood flow (*BF*), blood pressure (*BP*), and resistance (*Resist.*) shown as the per cent of change from base line following a single injection of 15 mg. of methadone (0.75 c.c.) into the fetal sagittal vein. Time on abscissa is in minutes.

bradycardia was noted in each experiment following the injection of methadone. Recovery from the bradycardia was prolonged beyond recovery of the cerebral cardiovascular parameters studied. In three of the eight experiments, as shown in Fig. 1, the heart rate never returned to base-line rates.

Fetal EEG. The effect of an acute bolus of methadone on fetal brain function in terms of the EEG was dose related. An injection of less than 10 mg. of methadone failed to reveal any visible change in the fetal EEG while cardiovascular alterations were evident. Plasma methadone concentrations were generally less than 0.05 mg. per cent with an injected dose of less than 10 mg. of methadone. With an injection of 10 to 15 mg. of methadone, plasma methadone concentrations were 0.05 to 0.65 mg. per cent and a decrease in amplitude, a dropout of faster frequencies, and a general slowing of the EEG were noted (Fig. 2). An injection of 20 mg. of methadone resulted in plasma methadone concentrations generally greater than 0.065 mg. per cent and rapid changes in the EEG (Fig. 3). Rapid slowing of the EEG with dropout in faster frequencies and decrease in amplitude preceded the onset of an isoelectric (flat) EEG. Recovery occurred by similar progression of activity and frequency as the plasma methadone concentration fell below 0.065 mg. per cent. No significant change in cerebral metabolism was noted following injection of methadone.

Comment

The absorption, the distribution, and the fate of methadone have been investigated extensively in recent years because of its accepted usage in detoxification and maintenance programs for heroin addicts. Following a single oral dose of 15 mg. of methadone, a mean peak plasma concentration of 0.0074 mg. per cent was found at four hours. The plasma concentration of methadone declined over the next 20 hours to a mean concentration of 0.0029 mg. per cent.[11] Similar studies were performed by the same group of investigators on methadone-maintenance subjects who had been receiving a daily oral dose of 100 or 120 mg. The predose mean plasma concentration of methadone of 0.048 mg. per cent increased to a peak concentration at four hours of 0.086 mg. per cent which slowly declined over 24 hours to a mean predose value.[12] Plasma methadone concentrations associated with death which have been reported as medical examiner cases ranged

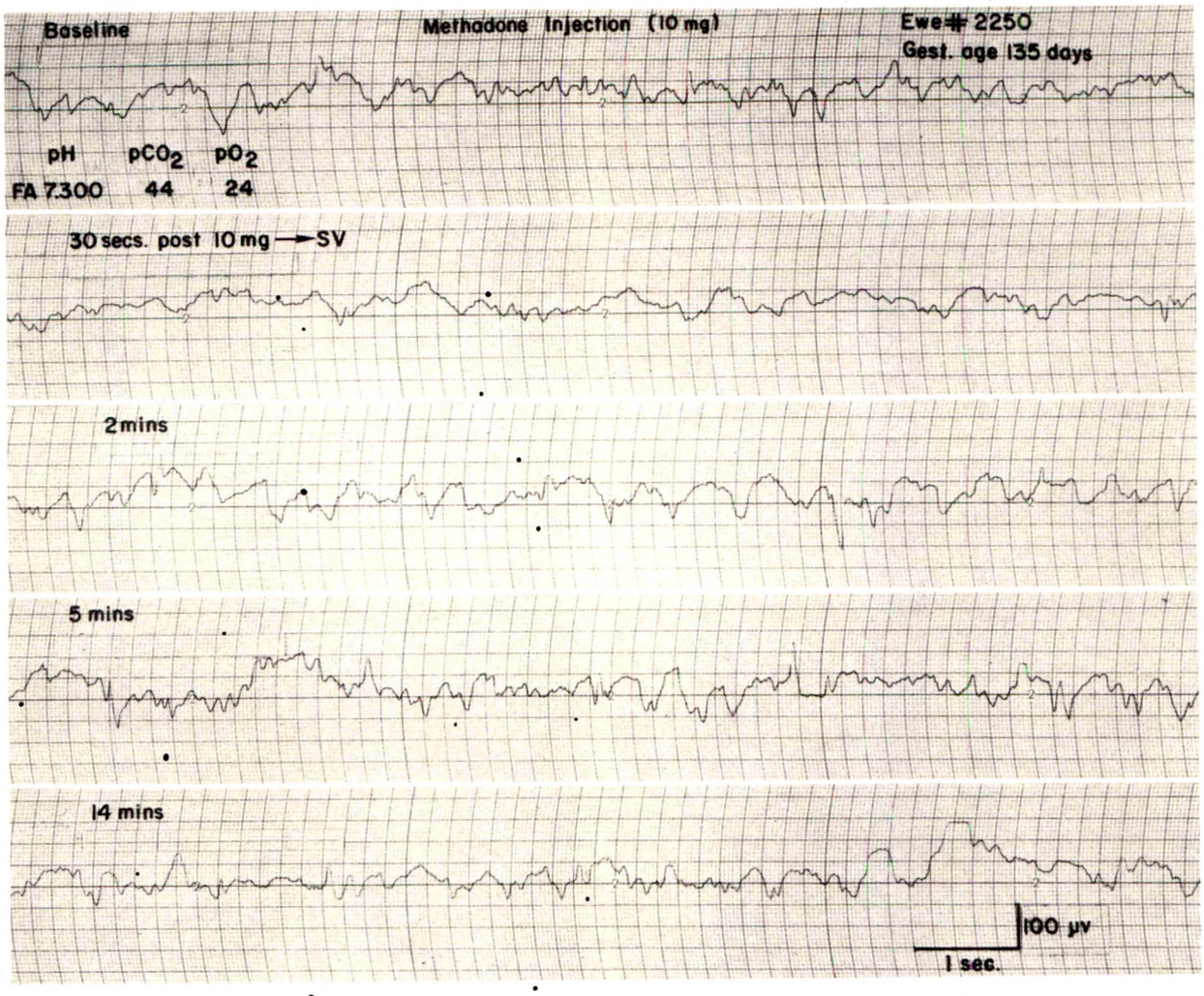


Fig. 2. Fetal EEG record following a single injection of 10 mg. of methadone (0.50 c.c.) into the umbilical vein (*SV*). *FA* = Carotid artery. Base line heart rate, 150 beats per minute; 30 seconds, 130; 2 minutes, 120; 5 minutes, 135; 14 minutes, 160.

from 0.06 to 0.18 mg. per cent, with general toxic levels reported as greater than 0.10 mg. per cent.[10, 13, 14] Blinick and associates[5] studied a group of pregnant patients in a methadone-maintenance program and reported observations of simultaneously obtained maternal plasma and amniotic fluid methadone concentrations. In maternal plasma, the concentration of methadone varied from 0.014 to 0.048 mg. per cent with a concentration in amniotic fluid varying from 0.010 to 0.045 mg. per cent. The amniotic fluid/maternal plasma ratio averaged 0.73 with a range of from 0.39 to 1.56. Fetal plasma levels of methadone were not obtained in the latter study. It can be concluded that methadone traverses the human placenta and membranes and that significant concentrations of methadone can be expected within the fetus and amniotic fluid. The failure to observe significant transport of methadone from mother to fetus or fetus to mother with high plasma concentrations of methadone in these sheep studies is not readily explained other than by morphologic species differences or poor experimental design. Further studies are required to elucidate this question.

Studies on the penetration of methadone across the blood-brain barrier have been conducted in rats following a common carotid artery injection of methadone.[6] The results of these studies indicated a very rapid uptake of methadone, approaching 42 per cent of the injected dose within 30 seconds. These uptake rates obtained from carotid injection could be expected to be similar to uptake rates following systemic administration as protein-binding studies

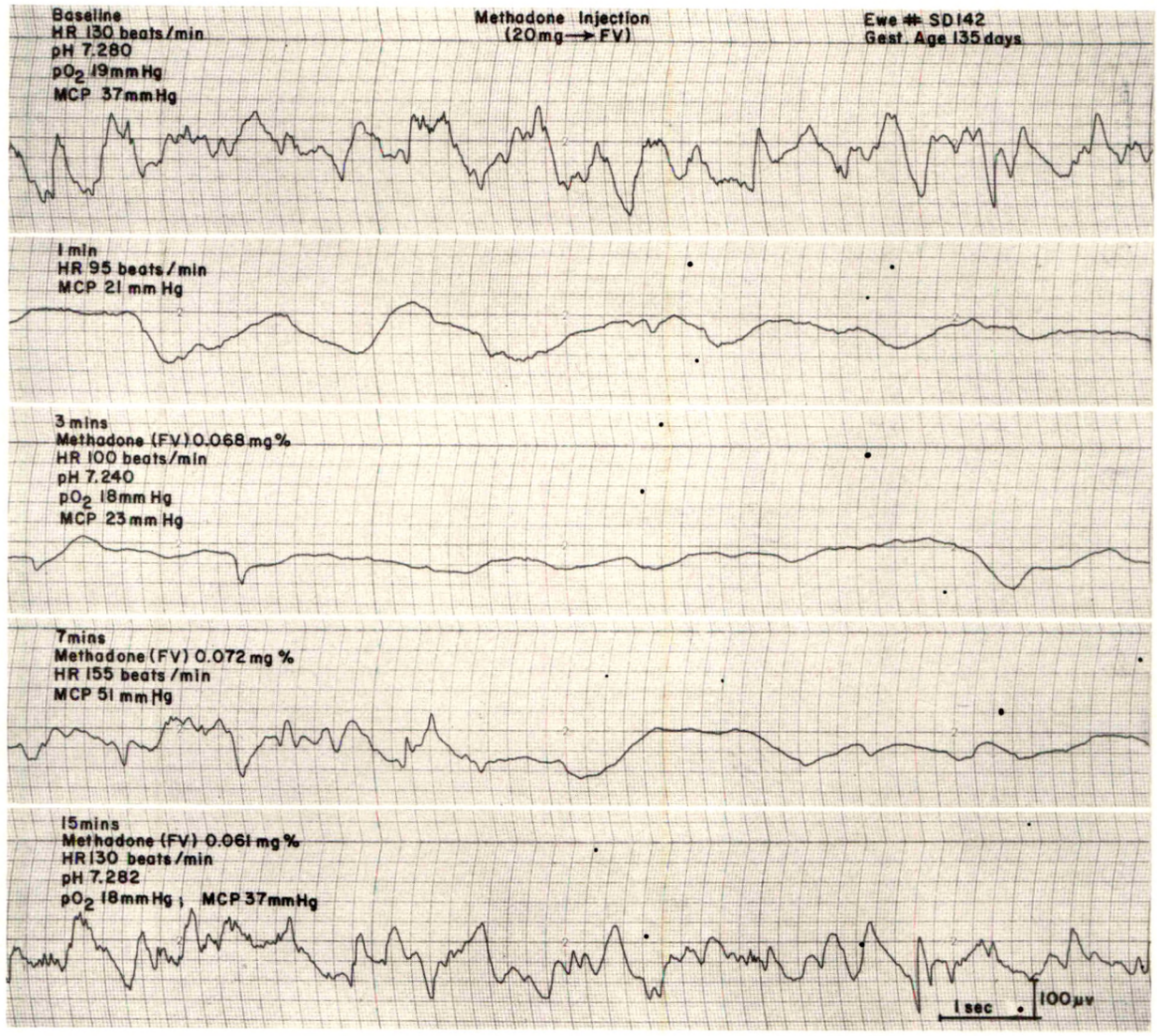


Fig. 3. Fetal EEG record following a single injection of 20 mg. of methadone (1.0 c.c.) into the umbilical vein (*FV*). pH and Po_2 are from the carotid artery. Plasma methadone is from the *FV*. *MCP* = Mean carotid pressure.

have indicated that 33 to 82 per cent of methadone is unbound in plasma.[15] The very rapid onset of both cardiovascular and EEG changes observed in the present experiments is consistent with the observations of rapid tissue uptake of methadone. The time-dose—effect relationship that was observed in these experiments is consistent with human observations where a direct time-dose relationship was shown for blood concentration of methadone with the physiologic parameter of pupillary constriction.[11]

While fetal bradycardia was noted with plasma methadone concentrations less than 0.05 mg. per cent, visible changes in the EEG did not occur until this concentration of methadone was obtained. The bradycardia was most likely the result of central vagal stimulation, but a direct myocardial effect cannot be excluded at this time. The slowing, dropout of faster frequencies, decrease in amplitude, and, on occasion, flattening of the fetal EEG are consistent with the increase in slow wave activity and decrease in fast wave activity seen by the investigators in the adult human subject.[16]

The data presented here leave unanswered the question of whether long-term administration of methadone during pregnancy could produce functional changes in the fetal central nervous system which would persist into the immediate and late neonatal period and possibly beyond. Adequate prospective

longitudinal studies must be performed before this critical question can be answered.

The authors wish to acknowledge the cooperation and assistance of Mr. Peter Lorenzo and the staff of the Animal Research Division of the Nassau County Medical Center in the performance of these experiments, and Dr. J. Bidouset and Mr. Tom Manning of the Toxicology Division, Nassau County Medical Examiners Office, for the performance of the methadone determinations.

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Fetal syphilis in the first trimester

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Evidence of first-trimester fetal syphilis was sought in the products of conception in a therapeutic abortion clinic. During two collection periods of one week, five patients with serologic and clinical data consistent with recent syphilitic infection were identified. Their conceptuses were carefully examined by silver and immunofluorescent stains for the presence of Treponema pallidum. *Two of these five conceptuses (9 and 10 weeks' gestation) were found to contain* T. pallidum *by these methods. The literature supporting the Langhans layer/five months' placental barrier theory is reviewed and discussed in view of these two first-trimester cases.*

THERE IS A widespread belief that the spirochetes of syphilis are unable to cross the placenta and infect the fetus until sometime after the fourth month of gestation. This belief has become widely accepted through textbooks of obstetrics,[1] pediatrics,[2] and pathology.[3] The evidence that led to this belief, though no longer cited, comes from several large series of fetal autopsies collected in the prepenicillin era.

Dippel,[4] in 1944, reported the results of 67 autopsies on the aborted or miscarried fetuses of luetic mothers. He also gathered the two older similar series of McCord[5] and Trinchese.[6] The combined cases of all three series included over two hundred infected fetuses. Dippel graphed the combined results of all three of their series of autopsied fetuses as the per cent of the abortions of luetic women found to be infected against the month of gestation. There was no case of an infected abortion before 18 weeks, and there were two infected abortions at 18 weeks; from 18 weeks the percentage of the aborted fetuses that were infected rose steadily to peak and plateau around eight months' gestation, when about 35 per cent of the conceptuses showed evidence of luetic infection at autopsy. The conclusion drawn was that spirochetes, although present and living in the mother, do not infect the fetus until after 18 weeks' gestation.

Dippel[4] reviewed the theories that had been proposed to explain this phenomenon. The earliest suggested that the increasing infection rate observed as gestation progresses was merely due to the increasing length of time that the fetus was exposed to passing spirochetes in the maternal blood. Trinchese[6] elaborated further, arguing that the incidence of fetal infection rose in direct proportion to the increasing surface area of the chorionic villi, as the placenta grew.

Neither of these theories was very popular since they both involved the assumption that fetal infection was a chance event made more probable later in gestation by increased exposure. This assumption, that infection was a chance event, failed to explain why, despite exhaustive efforts, no one had found a single infected abortus of less than 18 weeks' gestation. This led Beck and Daily[7] to offer another theory. They proposed a placental barrier. "About eight days after fertilization the developing ovum is implanted in the decidua. At this time it is surrounded by an active trophoblast which, for a considerable period, has the power of eroding the maternal tissues. Whether the erosive action is accomplished directly by the cells themselves or by some product of their activity, we may reasonably assume that it will have similar destructive effects upon any spirochetes that may cross its path. In addition to this possible spirocheticidal factor that may envelop the growing ovum, the developing trophoblast soon is differentiated into a layer of active Langhans cells. As long as the constituents of these layers are alive and vigorous, they no doubt act as an efficient barrier to the

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invasion of the spirochete. After the middle of pregnancy the Langhans cells become less and less evident . . . It is not surprising, therefore, that fetal infection is common after the fifth month."

Dippel[4] found this theory very appealing. Using his series of 67 fetuses of luetic mothers, he rated the histologic appearance of the Langhans layer in each placenta. He compared the percentage showing delay in regression of the Langhans layer in the infected group (18.75 per cent) to the percentage showing delayed regression in the noninfected group (50 per cent). He concluded ". . . that the Langhans cells do afford a measure of protection against maternal luetic infection . . ." and that there ". . . is no apparent explanation for the intrauterine protection other than the Langhans layer of chorionic epithelium."[4]

However, there were skeptics. McCord[5] (82 positive cases) had said that he ". . . could see no reason why the organisms of syphilis could not be found in early abortions . . ." and ". . . continued extensive work along this line . . ." but without finding a single earlier case.

Dorman and Sahyun[8] had reported a single case (in 1937) of an infected placenta in a four-month abortus, but their single case had been overlooked by Dippel as well as later reviewers.

Silverstein,[9, 10] in 1962, suggested as a result of work on the timing of immunogenesis in the fetus that the lack of histologic evidence of fetal infection ". . . does not represent the timing of the initial infection of the fetus, that is, of placental transmission of the organism . . ."[9] but rather ". . . the earliest lesions of congenital syphilis become apparent only after the development in the fetus of a functional immunologic apparatus and of the ability to mount a chronic inflammatory response."[10]

Stowens[11] lent support to this idea in his text by stating that in the rare cases where spirochetes were found in fetuses before the fifth month of gestation there were no signs of inflammatory response. However, evidence that refuted the theory of a placental barrier was not forthcoming, and soon after Dippel's[4] publication congenital syphilis became an increasingly rare disease.

Unfortunately, today, 30 years later, congenital syphilis is again of increasing interest as it is increasing in incidence. In 1975, we have an opportunity to examine this old problem in new ways. There are now available for the first time many very early and fresh abortuses from syphilitic women through therapeutic abortion clinics, as well as new techniques to ascertain the presence of spirochetes.

Material and methods

Tissue collection. Potentially syphilitic abortion material was collected by biopsying the products of all therapeutic abortions performed at the John Wesley County Hospital, Los Angeles, during two one-week collection periods. They were held until the results of the maternal Venereal Disease Research Laboratory (VDRL) and FTA-ABS serologic studies were available. All abortions performed at John Wesley County Hospital were in women who were clinically judged to be less than 12 weeks pregnant. They were all done by suction curettage into suction bags. The contents of the bags were examined immediately after the procedure. Villous tissue, if grossly evident, was washed in saline, and two biopsy specimens were taken. If fetal body parts were seen, they were biopsied as well, but in many cases (44 per cent) only placental tissue was identifiable.

The first specimen was fixed in 10 per cent formalin for examination by routine hematoxylin and eosin and silver stains. The second biopsy specimen was frozen in a beaker of 2-methyl butane chilled by suspending it over a container of liquid nitrogen. The tissue was then stored in a liquid nitrogen freezer for later use in immunofluorescent stains.

Silver stain. The silver stain employed in this study was the Warthin-Starry Stain, and the procedure was exactly as described in the manual of the Armed Forces Institute of Pathology.[12] The positive control tissue used for this stain was formalin-fixed infected rabbit testis.

Immunofluorescent stain. The immunofluorescent staining technique used in this study is an indirect stain utilizing the basic technology developed for the now routine FTA-ABS serologic test. In the FTA-ABS test, sera of unknown reactivity are placed on a smear of known *Treponema pallidum* (Nichols strain), and the sera are checked for the specific antibodies characteristic of syphilis. In this study the test was modified so that sera of known 4+ reactivity to *T. pallidum* were placed on tissue of unknown *T. pallidum* content to check for the presence of the organisms of syphilis. The details of the technique are analogous to those described in the United States Public Health Service *Manual of Tests for Syphilis,*[13] except for slide preparation and tissue handling.

Tissue for the immunofluorescent stain was held in liquid nitrogen. The day prior to staining, it was removed and, while still frozen, sectioned (4 μ thick) on a cryostat at −20° C. The sections were placed on FTA-ABS slides and allowed to melt and then to dry for a half hour. After drying, they were fixed in filtered acetone for 10 minutes and stored at 5° C.

The sera employed on all of our test sections was pooled sera from patients found to be 4+ reactive with the FTA-ABS test. This serum was diluted to its working titer and absorbed with FTA-ABS sorbent (a standardized extract of nonpathogenic treponemes with treponemal group antigens) exactly as described in the reference.[13]

The tissue sections at room temperature were then flooded with 0.03 ml. of the reactive absorbed serum and incubated in a moist chamber at 37° C. for 30 minutes. Slides were then rinsed and gently dried as described in the manual,[13] and 0.03 ml. of fluorescein-labeled caprine anti-human globulin was added to the slide. The fluorescein was prepared just as for FTA-ABS and diluted to its working titer on the day of use. The fluorescein conjugate was incubated on the slide for 30 minutes at 37° C. in a moist chamber. Rinsing was repeated, and the slides were mounted with a glycerine mounting medium and then examined immediately with a fluorescent microscope.*

The results of this technique were readable but had a significant amount of background immunofluorescence which made scanning the slides laborious. The technique was improved in repeated studies by the following changes: (1) Tissue sections once cut and dried were washed for half an hour in cold normal saline prior to acetone fixation to wash away some of the unbound globulins from maternal sera in the suction bag. Tissue was then dried again and acetone fixed as described above. This helped decrease nonspecific immunofluorescence and did not seem to affect the specific fluorescence of the spirochetes. (2) An attempt was made to further decrease nonspecific fluorescence by absorbing the 4+ reactive sera with normal VDRL-negative placental tissue of 10 weeks' gestation. This was done by mixing finely chopped, acetone-fixed trophoblastic tissue of 10 weeks' gestation with the 4+ reactive sera, incubating the mixture for 30 minutes at 5° C., and then removing the tissue. The serum was later reabsorbed with sorbent (Reiter treponemal extract) immediately prior to use, as described before. Although this technique further decreased the background fluorescence, it also seemed to decrease somewhat the specific brightness of the spirochetes as well.

Control slides for the immunofluorescent stain included three groups: (1) normal placental controls, (2) normal fetal controls, and (3) *T. pallidum* (Nichols strain) smear controls. Each of these three sets of controls was treated with: (1) reactive serum unabsorbed, (2) reactive serum absorbed, (3) normal serum, (4) sorbent alone, (5) phosphate-buffered saline alone.

Control Groups 1 and 2 provided a control for nonspecific fluorescence and artifacts. Control Group 3 provided a check for appropriate fluorescence and comparative morphology. Ideally, controls should include fresh frozen placenta, fetus, or even rabbit testis which is heavily infested, rather than a *T. pallidum* smear. However, these fresh, highly infectious materials were not available.

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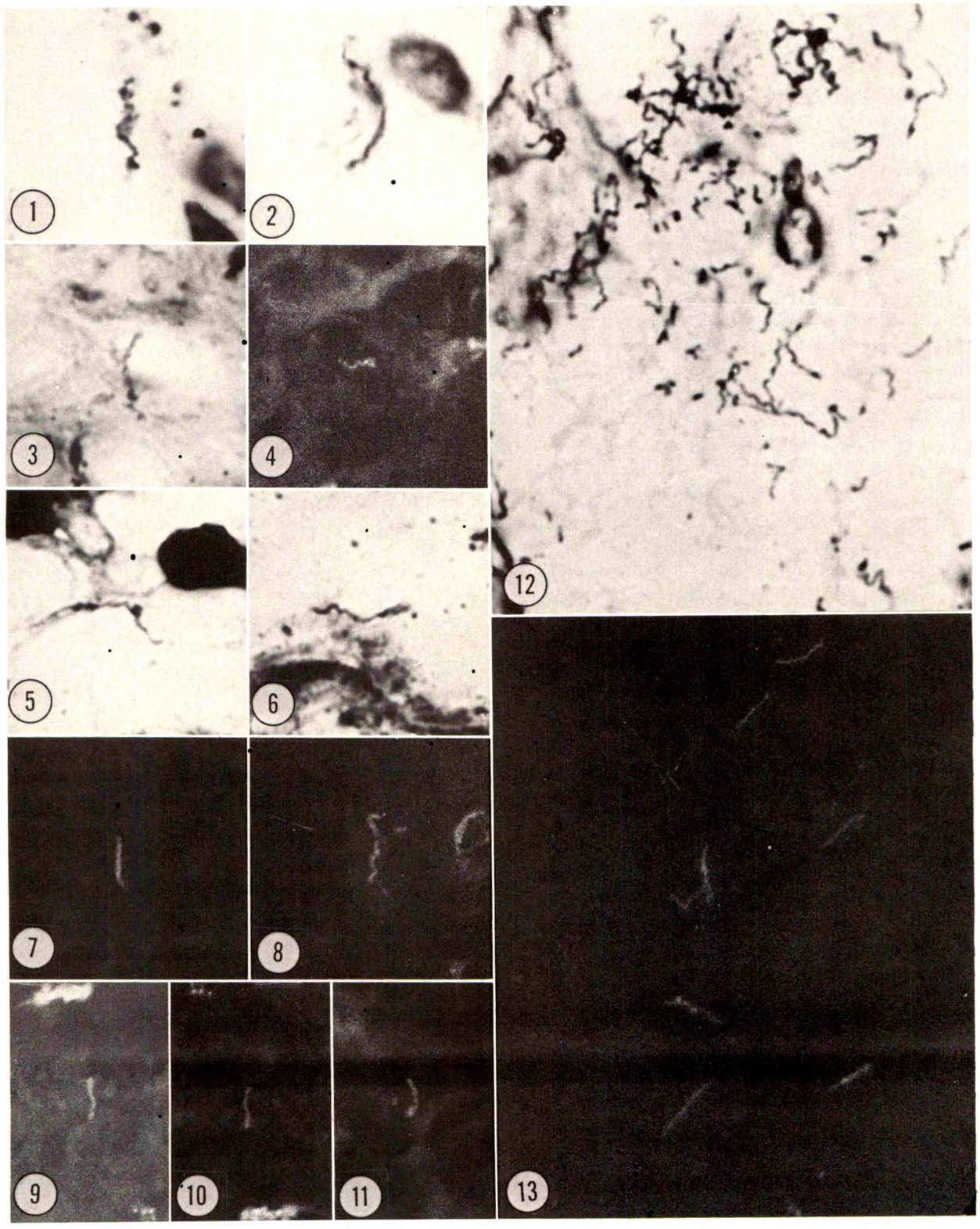
Table I. Serologic studies and clinical histories

Case no.	*VDRL*	*FTA*	*Maternal history*
1	+	+	Untreated, no prior history
2	+	Borderline	Untreated, no prior history
3	+	+	Untreated, no prior history
4	+	+	Untreated, no prior history
5	+	Borderline	Untreated, no prior history
6	Rough	+	Treated, congenital
7	Weakly reactive	−	No prior history, false positive VDRL
8	+	+	Treated, latent

Results

Tissue collection. In the two tissue collection periods of one week, the biopsy of 114 abortuses, all less than 12 weeks' gestation, was accomplished. Of these 114 abortions, there were eight from mothers with nonnegative VDRL tests. Seven of these women had non-negative FTA-ABS serologic tests as well. The results of the serologic studies and clinical histories with regard to syphilis are shown in Table I. None of these women gave a history of chancre, and none had visible lesions at the time of curettage. None had been told that they had syphilis in the past, except for the treated cases, Nos. 6 and 8. Several had children previously, but the results of previous antenatal serologic tests were unavailable to us. None had children with diagnosed congenital syphilis. The first five patients were judged to be possibly infected, and their abortuses were examined by silver and immunofluorescent stains. Each case was paired with the next consecutive normal case as a normal control.

Silver stains. Silver stains were unremarkable in all of our normal control cases and in three of our five VDRL-positive cases. Rabbit testis controls revealed abundant classic spirochetes 7 to 15 μ long with tight even spirals. Two of the five VDRL-positive cases (Cases Nos. 4 and 5) revealed small silver-positive



Figs. 1-13. For legend, see opposite page.

undulating elements of the appropriate length or shorter (4 to 10 μ). These suggested fragments of treponemes. Prolonged search yielded a few classic-appearing spirochetes (Figs. 1 to 3, 5, and 6) when compared to morphologically similar spirochetes from the rabbit testis control (Fig. 12). The spirochetes, although rare, were found scattered throughout the fetal and placental tissue in Case No. 4 but in only one small area of the fetal tissue in Case No. 5.

Immunofluorescent stains. Immunofluorescent stains revealed no spirochete-like fluorescent forms in either the normal placenta or normal fetal controls and gave 4+ brightness to the spirochetes on the Nichols treponeme control smears. No spirochetes were found in the tissues from Case No. 1, 2 or 3. Case No. 4 had rare spirochete-like fragments in the fetus and one rather characteristic form seen in Fig. 4. No treponemes were seen in the placenta from No. 4. In Case No. 5, the spirochetes were more uniform in size and somewhat more spiral in form. Spirochetes were found mainly in fetal tissues but also in the placenta (Figs. 7 to 11).

The immunofluorescent stain, as one can see from the Nichols control treponemes (Fig. 13), outlines the spirochetes in a soft glow which makes their morphology particularly difficult to appreciate in a black-and-white photograph. However, the size and shape as well as the binding of the specific sera make them characteristic.

Case No. 4 was a 33-year-old, Spanish-speaking woman, gravida 5, para 3, therapeutic abortions 2, who was 10 weeks' gestation by dates and uterine sounding at the time of curettage. There was no known prior history of syphilis.

Case No. 5 was a 24-year-old, Spanish-speaking woman, gravida 3, para 2, therapeutic abortions 1. She was nine weeks' gestation by dates and sounding at the time of curettage. There was no prior history of either chancre or positive serology. Her youngest child was 14 months old at the time of the curettage, and both her children were alive and well.

Comment

Spirochetes were found in the products of conception in two of our five cases (40 per cent) where the maternal serologic tests and histories were suggestive of recent syphilitic infection. These fetuses were nine and 10 weeks of gestation by dates and physical examination prior to curettage. Although our series is small, the rate of infection found in our untreated VDRL-positive women is strikingly similar to the rate found by Dippel,[4] McCord,[5] and Trinchese[6] later in pregnancy.

The question arises immediately as to why Dippel,[4] McCord,[5] and Trinchese,[6] in their large combined series, failed to find evidence of fetal infection at less than 18 weeks' gestation. We would suggest the following possible explanations. The first possibility is their method of case selection. Their three series contained only material from spontaneous abortions and stillbirths. Syphilis, as it has long been recognized, is not a cause of first-trimester abortion. Dippel's[4] series of 67 abortions of syphilitic women had only 12 cases less than 18 weeks' gestation (all negative). None of these was less than 14 weeks' gestation. McCord,[5] in his series of 200 autopsies of aborted fetuses from syphilitic mothers, had only one case of less than 18 weeks gestation, which was a 100 gram (approximately 16 weeks) fetus, also negative. Trinchese[6] unfortunately does not reveal the total number of fetuses that were under 18 weeks' gestation. Thus, although there were well over 200 cases in their combined series, only

Fig. 1. Placenta from Case No. 4. (Warthin-Starry silver stain. Original magnification ×2,500.)
Fig. 2. Fetus from Case No. 4. (Warthin-Starry silver stain. Original magnification ×2,500.)
Fig. 3. Placenta from Case No. 4. (Warthin-Starry silver stain. Original magnification ×2,500.)
Fig. 4. Fetus from Case No. 4. (Immunofluorescent stain. Original magnification ×1,000.)
Fig. 5. Fetus from Case No. 5. (Warthin-Starry silver stain. Original magnification ×2,500.)
Fig. 6. Fetus from Case No. 5. (Warthin-Starry silver stain. Original magnification ×2,500.)
Fig. 7. Fetus from Case No. 5. (Immunofluorescent stain. Original magnification ×1,000.)
Fig. 8. Fetus from Case No. 5. (Immunofluorescent stain. Original magnification ×1,000.)
Fig. 9. Fetus from Case No. 5. (Immunofluorescent stain. Original magnification ×1,000.)
Fig. 10. Fetus from Case No. 5. (Immunofluorescent stain. Original magnification ×1,000.)
Fig. 11. Placenta from Case No. 5. (Immunofluorescent stain. Original magnification ×1,000.)
Fig. 12. Rabbit testis control. (Warthin-Starry silver stain. Original magnification ×2,500.)
Fig. 13. Nichols treponeme smear. (Immunofluorescent stain. Original magnification ×1,000.)

a handful of those examined were less than 18 weeks' gestation.

Another problem in evaluating these spontaneously aborted specimens is that they were not always fresh. Many cases—50 per cent of Trinchese's earliest cases, an unknown per cent of McCord's cases, and 38.8 per cent of Dippel's cases—were macerated. The effect of intrauterine death and maceration on spirochetes is unknown. McCord[14] believed that fetal death decreased the likelihood of finding treponemes in his 200 cases. Morison[15] claimed the opposite, citing evidence that intrauterine death makes finding spirochetes easier as the anerobic conditions in utero foster the organisms' reproduction prior to expulsion. In any event, both maceration and the freshness of the specimen prior to fixation probably have effects on the ability of the examiner to demonstrate these sensitive organisms in tissue. Our specimens were all fresh and fixed minutes after curettage.

Last, none of these authors described how much effort they expended in the search for spirochetes in the youngest fetuses. Locating the organisms in our nine and 10 week fetuses was difficult. First, the spirochetes were not visibly destructive and did not cause surrounding necrotic lesions. Second, in these very young fetuses, there was no associated inflammatory response. Third, in our cases, the organisms were very scarce. It is interesting to note that the earliest infected case previously described in the literature was in the placenta from a four-month-old fetus reported by Dorman and Sahyun.[8] Their method of examination was exhaustive; they utilized five blocks of placental tissue and both hematoxylin and eosin and silver stains. They stated that four of the five blocks could easily be free of spirochetes and yet a focus of infection be found in the fifth. Fourth and last, as observed by other workers, the spirochete is perhaps somewhat variable in morphology by silver stain in tissues. Both our infected cases contained many nonspiral bodies which, although not present in the negative controls, were not classic spirochetes. McCord[5] said, "The organisms of syphilis are a puzzle to me . . . Sometimes the organisms are long, then short, a few spirals, many spirals, thin, others thick. . . . Fragmented organisms are often seen in macerated tissues, they appear distributed in showers."[5]

The use of indirect immunofluorescent stains with sera specifically 4+ reactive to *T. pallidum* allowed us to confirm the presence of the classic spirochetes as well as lend support to McCord's observations about the possible nature of these "fragments." It could well be that these silver-positive fragments are parts of the spirochetes themselves, for with the immunofluorescent staining technique there are similar fragment-like forms which specifically bind the anti-treponemal antibodies and fluoresce.

The Langhans layer placental barrier, as conceived by Beck and Daily,[7] does not act to protect the young fetus from invasion by spirochetes. The Langhans layer in these two infected specimens was typically active for 10 weeks' gestation.

Although no physical barrier exists (for there were organisms in both mother and fetus), it is interesting to note that the fetal organisms are so rare. Certainly spirochetes rarely, if ever, overwhelm the early fetus, causing intrauterine death and expulsion, as is so common in the last trimester. It has been shown repeatedly that syphilis is not a cause of first-trimester losses.

Whatever the factors responsible for the attenuated virulence of early fetal infection, the often-attenuated lues in the pregnant mother herself, or the claimed protective effect of pregnancy on the late complications of neurosyphilis,[7] they seem to have little to do with mechanical barriers and more to do with the as yet unknown biochemical requirements for this sensitive organism's growth and reproduction and/or its little understood immunologic status in pregnancy.[16, 17]

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The relationship between premature rupture of the membranes and the respiratory distress syndrome

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The records of 212 patients of 36 weeks' gestational age or less were reviewed to study the relationship between premature rupture of the membranes (PRM) and the development of the respiratory distress syndrome (RDS). PRM greater than 16 hours resulted in a statistically significant decrease of RDS in neonates of 32 weeks' gestational age or less but not in the group from 32 to 36 weeks' gestational age. However, survival was significantly improved with PRM greater than 16 hours in the latter group but not in the former. A possible explanation for this observation is offered. Black patients had a higher over-all infant mortality rate than white patients, and, although the incidence of RDS is similar in both races, it may be a more lethal condition in black patients. There was no significant difference noted between female and male infants. Sepsis was responsible for only seven deaths in this series, and three of those cases could not be etiologically related to prolonged PRM.

THE PROBLEM OF managing a patient of 36 weeks' gestational age or less who presents with premature rupture of the membranes (PRM) is very difficult. The choice often seems to lie between rapid delivery to avoid infection, with the attendant risk of death due to prematurity, versus watchful waiting in an attempt to "buy some time" and allow maturation to continue, while risking the possibility of chorioamnionitis and secondary neonatal sepsis.

Respiratory distress syndrome (RDS) is the major cause of death in premature infants in the United States today. Several papers recently have suggested that there is a decreased incidence of RDS in premature infants whose mothers have PRM for more than 16 to 24 hours.[1–5] However, these studies have not clearly demarcated differences in fetal outcome when the "premature infants" were further subdivided into gestational age groups, and racial differentials have not been described. Recognizing that the outcome of a 35 week gestation with PRM might well be different from that of a 30 week gestation with membranes ruptured for the same number of hours, we retrospectively studied all the infants admitted to our Newborn Special Care Unit (NSCU) in a 14½ month period whose gestational ages were 36 weeks or less.

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Methods

A retrospective study was performed on the records of all infants of less than 36 weeks' gestational age admitted to the Yale–New Haven Hospital (YNHH) NSCU between January 1, 1974, and March 15, 1975. At the YNHH, all babies who are born at 36 weeks' gestational age or less are automatically admitted to the NSCU. This eliminates any bias which might have been responsible for the selection of those infants who were transferred to the unit from other hospitals. Therefore, we considered the group born at YNHH to be representative of the "premie" population served locally by our hospital and separated it from the transfer group for the purpose of data analysis. In this series of the 212 admissions to the NSCU, 127 were born at YNHH and 85 were transferred from other institutions.

Table I. RDS versus gestational age and hours of PRM

RDS	*Group A (<32 weeks)*				*Group B (32-36 weeks)*			
	PRM less than 16 hours	*PRM 16-24 hours*	*PRM more than 24 hours*	*Total*	*PRM less than 16 hours*	*PRM 16-24 hours*	*PRM more than 24 hours*	*Total*
YNHH								
Absent	21	6	10	37	34	6	12	52
Present	22	1	4	27	8	1	2	11
Total	43	7	14	64*	42	7	14	63†
Transfers								
Absent	13	1	11	25	8	1	2	11
Present	24	—	6	30	16	2	1	19
Total	37	1	17	55‡	24	3	3	30§

*P = 0.07.
†Not statistically significant.
‡P = 0.055.
§Not statistically significant.

Gestational age scoring was performed independently by two observers on all infants with the use of the method of Dubowitz and associates.[6] The age determined was then checked against the Colorado intrauterine growth curve for weight, height, and head circumference.

PRM was defined as rupture of the membranes prior to the onset of labor. The duration of rupture of the membranes was considered to be that period from the time of their rupture until delivery.

The diagnosis of RDS was made on the basis of: (1) characteristic radiographic appearance, (2) blood gas monitoring for progressive acidosis associated with an increase in $F_{I_{O_2}}$ requirements, and (3) classical clinical manifestations including tachypnea, cyanosis in room air, intercostal retractions associated with persistent expiratory grunts, and decreased or absent breath sounds on auscultation.

The decisions to institute a work-up for sepsis and to give broad-spectrum antibiotic coverage were determined by both perinatal history and clinical course. The following scoring system has been devised at the Yale–New Haven Hospital to take these factors into account: birth weight <2,500 grams, 1; PRM >12 hours (premature infant) or >24 hours (term infant), 2; Apgar score at one minute <7, 2; maternal fever >38° C., 1; labor >12 hours, 2.

The infant's score is the numerical value of a composite of all positive findings. If the score is 2 or greater, a gastric aspirate is obtained. A negative aspirate (less than 5 polymorphonuclear leukocytes per high power field) is not followed by further work-up unless the infant's clinical condition warrants it. A positive aspirate is followed by a blood culture. If the clinical condition is stable, no antibiotics are given unless positive cultures are obtained. However, if the infant's clinical picture is suggestive of sepsis, antibiotic therapy is commenced immediately after the appropriate cultures have been taken.

Probability values were determined by a two-tailed analysis of variance.

Results

RDS versus hours of PRM (Table I).

YNHH deliveries. Of the 127 neonates in this series, 38 (29.8 per cent) had RDS. Of the 85 infants born after PRM less than 16 hours, 30 (35.3 per cent) had RDS as opposed to 8/42 (19.0 per cent) with PRM more than 16 hours (chi-square value = 2.89; P = 0.09). Of the 38 cases of RDS, 30 (78.9 per cent) were associated with PRM less than 16 hours and 32/38 (84.2 per cent) were associated with PRM less than 24 hours.

In Group A (gestational age of 32 weeks or less), 27/64 (42.2 per cent) had RDS. Of those with PRM less than 16 hours, 22/43 (51.2 per cent) had RDS, as opposed to 5/21 (23.8 per cent) with PRM more than 16 hours (chi-square value = 3.28; P = 0.07).

In Group B (gestational ages of 32 to 36 weeks), 11/63 (17.5 per cent) had RDS. Of those with PRM less than 16 hours, 8/42 (19.0 per cent) had RDS as opposed to 3/21 (14.3 per cent) with PRM more than 16 hours. Only 17.5 per cent of this group had RDS, and the number of cases was too small to be meaningful. Of those cases which did exist, however, 8/11 (72.7 per cent) occurred in patients with PRM less than 16 hours.

Transfer patients. In this series 49/85 (57.6 per cent) of the neonates had RDS. Of those with PRM less than

Table II. Survival versus hours of PRM

	Group A (<32 weeks)				*Group B (32-36 weeks)*			
	PRM less than 16 hours	*PRM 16-24 hours*	*PRM more than 24 hours*	*Total*	*PRM less than 16 hours*	*PRM 16-24 hours*	*PRM more than 24 hours*	*Total*
YNHH								
Survived	22	2	7	31	38	7	14	59
Died	21	5	7	33	4	—	—	4
Total	43	7	14	64*	42	7	14	63†
Transfers								
Survived	25	1	14	40	19	3	3	25
Died	12	—	3	15	5	—	—	5
Total	37	1	17	55‡	24	3	3	30§

*Not statistically significant.
†P = 0.045.
‡Not statistically significant.
§P = 0.07.

16 hours 40/61 (65.6 per cent) had RDS as opposed to 9/24 (37.5 per cent) with PRM more than 16 hours (chi-square value = 4.47; P = 0.03). Of the 49 cases of RDS 40 (81.6 per cent) were associated with PRM less than 16 hours and 42/49 (85.7 per cent) were associated with PRM less than 24 hours.

In Group A, 30/55 (54.5 per cent) had RDS. Of those with PRM less than 16 hours, 24/37 (64.9 per cent) had RDS, as opposed to 6/18 (33.3 per cent) with PRM more than 16 hours (chi-square value = 3.67; P = 0.05).

In Group B, 19/30 (63.3 per cent) had RDS. Of those with PRM less than 16 hours, 16/24 (66.7 per cent) had RDS as opposed to 3/6 (50 per cent) of those with PRM of more than 16 hours. This does not attain statistical significance, but there were very few patients in this series with PRM more than 16 hours. Of the 19 cases of RDS which occurred, however, 16 (84.2 per cent) were in neonates with PRM less than 16 hours.

Survival versus hours of PRM (Table II).

YNHH deliveries. Of the 127 neonates in this series, 90 (70.9 per cent) survived.

In Group A, 31/64 (48.4 per cent) survived. Of those with PRM less than 24 hours, 24/50 (48 per cent) survived, as opposed to 7/14 (50 per cent) with PRM more than 24 hours. This is not statistically significant.

In Group B, 59/63 (93.7 per cent) survived. Of those with PRM less than 16 hours, 38/42 (90.5 per cent) survived as opposed to 21/21 (100 per cent) with PRM more than 16 hours (chi-square value = 4.03; P = 0.04).

Transfer patients. Of the 85 neonates in this series, 75 (88.2 per cent) survived.

In Group A, 40/55 (72.7 per cent) survived. Of those with PRM less than 24 hours, 26/38 (68.4 per cent) survived, as opposed to 14/17 (82.3 per cent) with PRM more than 24 hours. This is not statistically significant (chi-square value = 1.96; P = 0.17).

In Group B, 25/30 (83.3 per cent) survived. Of those with PRM less than 16 hours, 19/24 (79.2 per cent) survived, as opposed to 6/6 (100 per cent) with PRM more than 16 hours (chi-square value = 3.38; P = 0.07).

Racial differences in RDS and survival. Only 7/85 (8.2 per cent) of the transfer patients were black as opposed to 32/127 (25.2 per cent) of those born in YNHH. The following data refer to the latter group.

Nineteen of the 32 black infants (59.4 per cent) and 71 of the 95 white infants (74.7 per cent) survived (chi-square value = 3.53; P = 0.06). Of the black infants 9/32 (28.1 per cent) had RDS, and of those 7/9 (77.8 per cent) died. Of the white infants 29/95 (30.5 per cent) had RDS, and of those 14/29 (48.3 per cent) died. The difference in the mortality rate associated with RDS is statistically significant (chi-square value = 3.75; P = 0.05). Of the black infants who died, 7/13 (53.8 per cent) had RDS while 14/24 (58.3 per cent) of the white infants who died had RDS.

Sex differences in RDS and survival. Of the 127 neonates born at YNHH, 71 (55.9 per cent) were male. Of the male infants, 47/71 (66.2 per cent) survived as opposed to 43/56 (76.8 per cent) of the female infants. This is not statistically significant (chi-square value = 2.25; P = 0.13) but is consistent with the trend described by Naeye and associates.[7] Of the 71 male infants, 22 (31.0 per cent) had RDS, and 16/56 (28.6 per cent) of the female infants had RDS. Of the male infants who died, 13/24 (54.2 per cent) had RDS while

Table III. Primary causes of death

With RDS		*Without RDS*	
Main cause of death	*No.*	*Main cause of death*	*No.*
Born at YNHH (37 infants)			
RDS	9	Group B strep. sepsis	3
Intraventricular hemorrhage	3	Immaturity	3
Erythroblastosis	3	Asphyxia	3
Necrotizing enterocolitis	3	Necrotizing enterocolitis	2
Sepsis (not Group B strep.)	1	Congenital heart disease	2
Disseminated intravascular coagulation	1	Other lethal anomaly	1
Asphyxia	2	Intraventricular hemorrhage	1
Total	22		15
Transfers (20 infants)			
RDS	8	Group B strep. sepsis	2
Intrapulmonary hemorrhage	3	Immaturity	1
Intraventricular hemorrhage	2	Other lethal anomaly	1
Sepsis (not Group B strep.)	1	Necrotizing enterocolitis	1
Disseminated intravascular coagulation	1		5
Total	15		

8/13 (61.5 per cent) of the female infants who died had RDS.

The transfer patients were not studied in this category.

Gastric aspirates, blood cultures, and survival versus hours of PRM.

YNHH deliveries. Gastric aspirates were positive in 4/51 (7.8 per cent) of neonates with PRM less than 16 hours, 10/14 (71.4 per cent) with PRM of 16 to 24 hours, and 21/26 (80.8 per cent) with PRM more than 24 hours. This is statistically significant (chi-square value = 46.29; P = 0.001).

In the entire series of 127 patients, only 10 (7.9 per cent) had positive blood cultures, and 5 of these were in patients with PRM less than 16 hours. Fifty-five patients were treated with antibiotics, despite the relative paucity of positive cultures. Of the 127 neonates in this series, 4 (3.1 per cent) died from sepsis, and one of these was an infant who also had RDS. The remaining three deaths were caused by Group B streptococcus and were associated with PRM of 6, 22, and 120 hours. Another infant in this series who had a positive blood culture for Group B streptococcus and PRM for 21 hours survived.

Transfer patients. Gastric aspirates were positive in 1/30 (3.3 per cent) of neonates with PRM less than 16 hours, 3/4 (75 per cent) with PRM of 16 to 24 hours, and 17/20 (85 per cent) with PRM more than 24 hours. This is statistically significant (chi-square value = 36.05; P = 0.001).

In this series of 85 patients, 6 (7.1 per cent) had positive blood cultures, all of which were in patients with PRM less than 16 hours. Forty-seven patients were treated with antibiotics. Three of the neonates died from sepsis, and one of these also had RDS. The other two deaths were secondary to Group B streptococcus and were associated with PRM of 0 and 6 hours. These figures, as well as those from the YNHH group, suggest that PRM plays a minor role in the pathogenesis of Group B streptococcal perinatal sepsis. This is consistent with the data reported by Echeverria.[8]

Causes of death (Table III). Of the deaths in the YNHH group, 22/37 (59.5 per cent) occurred in patients who had RDS. Except for the five cases of erythroblastosis fetalis and asphyxia, all of the remaining listed causes of death in the RDS group could be related to complications of the disease itself or to therapeutic modalities used for treatment. Indwelling umbilical arterial lines are associated with an increased incidence of necrotizing enterocolitis, sepsis, and disseminated intravascular coagulation, while respirators create an increased risk of intraventricular and intrapulmonary hemorrhage.

Of the deaths in the transfer group, 15/20 (75 per cent) were in patients who had RDS. All of the deaths in the RDS group were directly attributable to respiratory failure secondary to progression of the disease or to complications known to be associated with it.

When both groups are combined, 17/57 (29.8 per cent) of the total deaths in this series were directly due to RDS. In addition, 8/57 (14 per cent) occurred in patients with RDS and were caused by conditions known to be complications of the disease, while an additional 7/57 (12.3 per cent) may have been due to therapeutic modalities used for treatment. Thus, it is theoretically possible that 32/57 (56.1 per cent) of the

deaths in this series could have been avoided if RDS had been eliminated. With this in mind, prevention of the disease takes on added significance.

In the combined series, there were 20 deaths in neonates who did not have RDS. Seven of these deaths (35 per cent) were due to immaturity or asphyxia and occurred in neonates who were all less than 26 weeks' gestational age. Four of the deaths (20 per cent) were caused by severe congenital anomalies, and 5/20 (25 per cent) were caused by Group B streptococcal sepsis. The remaining 4/20 (20 per cent) were secondary to necrotizing enterocolitis or intraventricular hemorrhage.

Comment

The currently accepted cause of RDS is a deficiency of pulmonary surfactant.[9] The latter is a surface-active phospholipid-protein complex which reduces surface tension on expiration, thereby preventing alveolar collapse. It is thought that pulmonary surfactant is produced by Type II alveolar cells, which begin to appear at 22 to 24 gestational weeks in the human fetus. Dipalmitoyl lecithin, a surface-active phospholipid, is a major component of surfactant. By methanol extraction, cold acetone precipitation, and thin-layer chromatography, Gluck and colleagues[10] have been able to demonstrate that amniotic fluid concentrations of both surface-active lecithin and non–surface-active sphingomyelin begin to rise at 24 to 26 weeks of gestation. A spurt in lecithin production occurs at 35 weeks while sphingomyelin concentration remains stable (or possibly falls). This results in a marked rise in the lecithin-to-sphingomyelin (L/S) ratio, and, when it exceeds a value of 2, in almost all cases the fetus has enough surfactant present for protection from RDS in the neonatal period.[11] The understanding of this phenomenon and the ability to measure L/S ratios with a relatively simple laboratory procedure have given obstetricians a valuable tool to assess fetal maturity.

Glucocorticoids have been injected into rabbit,[12] monkey,[13] and lamb fetuses[14] and have been shown to cause accelerated pulmonary maturity. This was evidenced by alveolar stability and survival of premature neonates at gestational ages when control animals were incapable of survival and had no appreciable amount of surfactant present. The suggestion was forthcoming that adrenal steroids could accelerate fetal maturity. Spellacy and co-workers[15] demonstrated that women between 28 and 32 weeks gestational age who were given an oral glucocorticoid for two weeks had significantly greater rises in L/S ratios than control subjects. Liggins and Howie[16] reported a decreased incidence of RDS in premature infants of less than 32 weeks whose mothers had received intramuscular betamethasone 24 hours prior to delivery, as compared to a group of control subjects. Murphy[17] reported that infants with RDS had lower cord blood levels of both cortisol and cortisone than did matched control infants. On the other hand, Baden and associates[18] administered postnatal hydrocortisone or placebos to 44 infants with RDS within 24 hours of birth, and there was no appreciable difference in the outcome of the two groups. This last report is compatible with the animal and human data described above if the mode of action of corticosteroids in enhancing lung maturation is through their action as enzyme inducers. It has been suggested that phosphorylcholine glyceride transferase in fetal lung parenchyma is induced by glucocorticoids. This, in turn, results in the production of increased amounts of surface active lecithin via the cytidine diphosphate–choline pathway.[12]

Evidence is accumulating that PRM is associated with a lowered incidence of RDS in premature infants. In 1972, Alden and colleagues[1] demonstrated an increased survival rate in infants weighing less than 1,000 grams who had PRM. An analysis of the data presented for the survivors in this series indicates that those infants with PRM had less RDS than those who did not have PRM. Richardson and co-workers[2] reported an incidence of RDS of 64 per cent in 42 neonates with PRM ≤24 hours and 31 per cent in 22 neonates with PRM >24 hours. The mean gestational age of the first group was 32.4 weeks, and that of the second was 30.4 weeks. The over-all survival rates in the two groups were 67 and 64 per cent. Yoon and Harper,[3] in a retrospective study of 211 premature infants, reported a significantly lower incidence of RDS in infants with PRM more than 24 hours when compared with infants with PRM less than 12 hours. No data were presented relating to survival. Chiswick and Burnard[4] studied 118 infants born before 35 weeks and found that there was a reduced incidence of RDS with PRM more than 24 hours, as well as fewer associated deaths. Gluck and Kulovich[11] described 11 cases in which the L/S ratio in patients with PRM >24 hours was "accelerated" (defined as an L/S ratio of 2 or more which is identified before 35 weeks of gestation). Bauer and associates[5] studied 17 mother-infant pairs and found a lower incidence of RDS in infants born after PRM greater than 16 hours. All the neonates in this small series survived. A positive correlation between cortisol and total blood corticosteroid levels in the infants and duration of PRM was observed.

These data, along with the observations that heroin addiction,[19] degenerative types of maternal diabetes (D, E, and F),[20] severe toxemia,[20] placental insufficien-

cy,[20] maternal hemaglobinopathies,[20] and intrauterine infection[21] have been associated with accelerated pulmonic maturity, lend credence to the hypothesis that stress in utero is responsible for increased glucocorticoid production which, in turn, acts as an enzyme inducer in the fetal lung.

Our study was undertaken to assess the effect of PRM on the development of RDS and survival when a number of different parameters were considered. The results were as follows.

For both the YNHH and transfer series, PRM greater than 16 hours was associated with a decreased incidence of RDS in neonates of 32 weeks' gestational age or less (Group A). Our data suggest that this may also be true for the group between 32 and 36 weeks (Group B), but the numbers were too small to substantiate this.

In both series, Group B figures for survival were significantly better in those with PRM more than 16 hours as compared to those with PRM less than 16 hours. This could not be demonstrated in Group A. Because of insufficient numbers, however, it was not possible to further subdivide this group by the actual number of weeks of gestational age.

Both racial and sex data were examined only in the YNHH group to rule out any selection bias in the transfer patients. Black infants had a higher over-all mortality rate than white infants. Our data suggest that while the incidence of RDS is similar in black and white infants it is a more lethal condition in the former.

Female infants had higher survival rates than male infants, but this was not statistically significant. The incidence of RDS was similar in both sexes.

There were only 16 positive blood cultures in this series of 212 patients and seven deaths related to sepsis. All septic deaths unassociated with concomitant RDS were caused by Group B streptococcus, and 3/5 of this small group had PRM for six hours or less.

Of all deaths in this series, 56.1 per cent were in patients who had RDS and died either from progression of the disease itself, known associated conditions, or complications arising from its therapy. Of the deaths in the group of neonates who did not have RDS, 35 per cent occurred in infants who were less than 26 weeks' gestational age.

We interpret these data to mean that there is a correlation between PRM more than 16 hours and a decreased incidence of RDS in neonates of 32 weeks' gestational age or less. This might also be true of those infants born between 32 and 36 weeks, but because of too few cases of RDS in the YNHH group and too few patients with PRM more than 16 hours in the transfer group our data cannot substantiate this.

We have been unable to demonstrate any significant improvement in survival with PRM more than 16 hours in neonates of 32 weeks' gestational age or less. In the group of 32 to 36 weeks' gestational age, however, survival does seem to improve with PRM more than 16 hours, and it is of interest that in our series there were no deaths at all in this category. The number of patients in this study was too small to permit a meaningful analysis of survival versus specific weeks of gestation. Extreme prematurity was responsible for 35 per cent of the deaths in the non-RDS group. With the elimination of deaths from severe congenital anomalies, one would expect to see increasing survival accompanying a decreased incidence of RDS with advancing gestational age beyond 28 weeks. This being true, the importance of RDS as a potentially avoidable cause of death becomes more evident.

The number of black patients in the YNHH series was small, but the data suggest that while the incidence of RDS is similar in black and white infants the condition is more lethal in the former. Additional studies are needed to confirm this impression.

Finally, an aggressive approach to possible fetal infection resulted in a relatively small number of deaths due to sepsis. All five septic deaths unassociated with RDS were caused by Group B streptococcus, and three of these were in patients with PRM of six hours or less. We conclude from this that it may be well worth the risk of waiting at least 16 hours before delivering a premature infant with ruptured membranes when weighing the dangers of neonatal infection versus RDS.

Addendum

A recent paper by Jones and co-workers[22] from the University of Colorado retrospectively analyzed 16,548 consecutive deliveries over a 12 year period for the association of PRM and RDS. Their study does not support the hypothesis that there is a lower incidence of RDS in infants born after prolonged PRM. However, gestational age was determined solely by dating from the last menstrual period. This is a method which can be significantly inaccurate. Also, a retrospective study over a 12 year period has the disadvantage of having many different individuals examine the infants. This may have resulted in some inconsistency in the diagnosis of RDS. Asphyxia can enhance the tendency to persistent fetal circulation which may present clinically and radiographically as RDS in the presence of normal lung maturation. Despite these difficulties, however, the Colorado study indicates that the relationship between RDS and PRM is far from settled. Carefully performed prospective studies with large numbers of patients are necessary to lay this question to rest.

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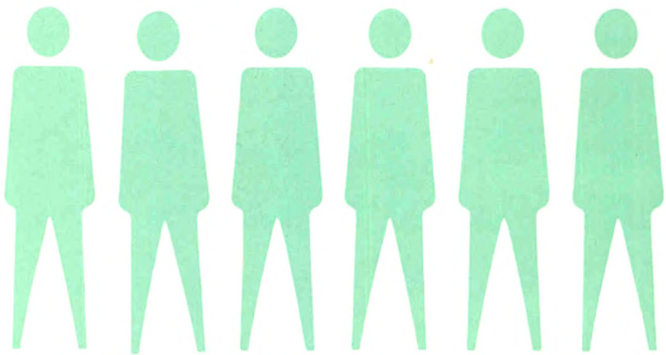
Please see summary of product information on the last page of this advertisement.

ROCHE

Straight talk about Gantrisin® in acute cystitis*

sulfisoxazole/Roche

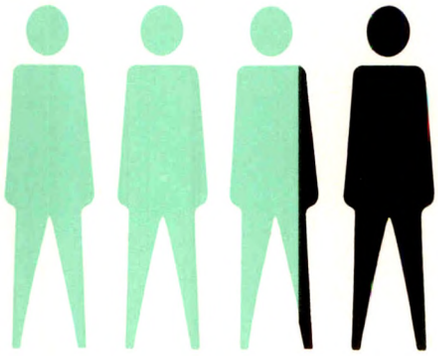
In a new study of Gantrisin (sulfisoxazole) in acute lower urinary tract infections, 89% of patients achieved sterile urine.†



No. of patients	Sterile urine	Clear culture (1000 or less organisms/ml urine)	Clear culture (10,000 or less organisms/ml urine)
251	89% (224)	90% (226)	92% (232)

Over the years Gantrisin (sulfisoxazole) has been the subject of continuing clinical research. There are many references which attest to its high degree of efficacy in acute cystitis. Now a new clinical study of acute lower urinary tract infection again demonstrates Gantrisin effectiveness. The pathogens causing these infections were *E. coli, Klebsiella-Aerobacter, P. vulgaris* and *P. mirabilis*, and the results—Gantrisin achieved 89% effectiveness.

*nonobstructed; due to susceptible organisms
†Data on file, Hoffmann-La Roche Inc., Nutley, New Jersey 07110



Gantrisin (sulfisoxazole) offers an established record of safety

Gantrisin introduced a new era of sulfonamide therapy coupling effectiveness and relative safety.[1-3] Side effects are few. In this study only 2% of the patients experienced side effects, which were mild. These included drowsiness, fatigue, rash, pruritus, diarrhea, nausea, vomiting, headache. The high solubility of Gantrisin minimizes risk of crystalluria and renal damage. However, as with any sulfonamide therapy, maintain adequate fluid intake and perform frequent urinalyses with careful microscopic examination.

1. Fass RJ, Klainer AS, Perkins RL: *JAMA 225*: 1509-1513, Sept 17, 1973
2. Eykyn SJ, Phillips I: *Practitioner 203*: 501-509, Oct 1969
3. Stamey TA: *Urinary Infections*. Baltimore, The Williams and Wilkins Co., 1972, pp. 280-282

Gantrisin (sulfisoxazole) costs less than most therapies

Few antibacterials can approach the economy of Gantrisin therapy. The average cost of 10-day Gantrisin therapy is less than 10 days of most other antibacterials.

Before prescribing, please consult complete product information, a summary of which follows:

Indications: Nonobstructed urinary tract infections (mainly cystitis, pyelitis, pyelonephritis) due to susceptible organisms.

IMPORTANT NOTE: *In vitro* sensitivity tests not always reliable; must be coordinated with bacteriological and clinical response. Add aminobenzoic acid to follow-up culture media. Increasing frequency of resistant organisms limits usefulness of antibacterial agents, especially in chronic and recurrent urinary infections. Maximum safe total sulfonamide blood levels, 20 mg/100 ml; measure levels as variations may occur.

Contraindications: Hypersensitivity to sulfonamides; infants less than 2 months of age; pregnancy at term and during the nursing period.

Warnings: Safety in pregnancy not established. Do not use for Group A beta-hemolytic streptococcal infections, as sequelae (rheumatic fever, glomerulonephritis) are not prevented. Deaths reported from hypersensitivity reactions, agranulocytosis, aplastic anemia and other blood dyscrasias. Sore throat, fever, pallor, purpura or jaundice may be early indications of serious blood disorders. CBC and urinalysis with careful microscopic examination should be performed frequently.

Precautions: Use cautiously in patients with impaired renal or hepatic function, severe allergy or bronchial asthma. Hemolysis, frequently dose-related, may occur in glucose-6-phosphate dehydrogenase-deficient patients. Maintain adequate fluid intake to prevent crystalluria and stone formation.

Adverse Reactions: *Blood dyscrasias:* Agranulocytosis, aplastic anemia, thrombocytopenia, leukopenia, hemolytic anemia, purpura, hypoprothrombinemia and methemoglobinemia; *Allergic reactions:* Erythema multiforme (Stevens-Johnson syndrome), generalized skin eruptions, epidermal necrolysis, urticaria, serum sickness, pruritus, exfoliative dermatitis, anaphylactoid reactions, periorbital edema, conjunctival and scleral injection, photosensitization, arthralgia and allergic myocarditis; *Gastrointestinal reactions:* Nausea, emesis, abdominal pains, hepatitis, diarrhea, anorexia, pancreatitis and stomatitis; *C.N.S. reactions:* Headache, peripheral neuritis, mental depression, convulsions, ataxia, hallucinations, tinnitus, vertigo and insomnia; *Miscellaneous reactions:* Drug fever, chills and toxic nephrosis with oliguria and anuria. Periarteritis nodosa and L.E. phenomenon have occurred. Due to certain chemical similarities with some goitrogens, diuretics (acetazolamide, thiazides) and oral hypoglycemic agents, sulfonamides have caused rare instances of goiter production, diuresis and hypoglycemia as well as thyroid malignancies in rats following long-term administration. Cross-sensitivity with these agents may exist.

Dosage: Contraindicated in infants under 2 months except in the treatment of congenital toxoplasmosis as adjunctive therapy with pyrimethamine.

Usual adult dosage—2 to 4 Gm initially, then 4 to 8 Gm/24 hrs in 4 to 6 doses. *Usual dosage for infants over 2 months and children*—½ 24-hrs dose initially, then 150 mg/kg/24 hrs in 4 to 6 doses; not over 6 Gm/24 hrs.

How Supplied: Tablets containing 0.5 Gm sulfisoxazole, white, scored—bottles of 100, 500 and 1000; drums of 5000; Tel-E-Dose® packages of 100; Prescription Paks of 100, available singly and in trays of 10.

ROCHE

Roche Laboratories
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Gantrisin®

sulfisoxazole/Roche

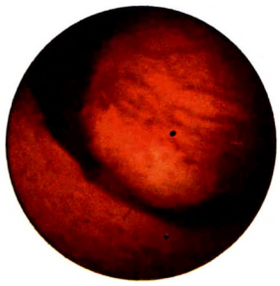
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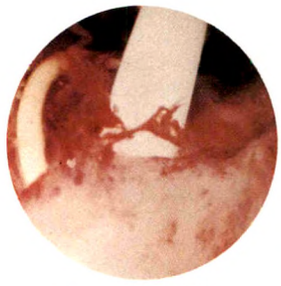
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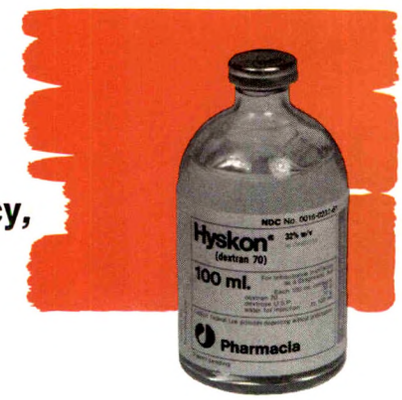
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- adds a new dimension of direct visualization, accuracy, and ease in the detection and treatment of intrauterine problems.[1-8]



For additional information on Hysteroscopy with **HYSKON®** **contact:** Medical Director, Pharmacia Laboratories, Piscataway, N.J. 08854

REFERENCES:

1. Cohen, M. R. Dmowski, W. P. Fert. & Steril. *24* (12):905, 1973.
2. Edstrom, K., Fernstrom, I.: Acta Obstet. Gynecol. Scand. *49*:327, 1970.
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Pharmacia

Pharmacia Laboratories
Division of Pharmacia Inc.
Piscataway, New Jersey 08854

PRESCRIBING INFORMATION

HYSKON® HYSTEROSCOPY FLUID

32% (W/V) dextran 70 in dextrose

DESCRIPTION: Hyskon Hysteroscopy Fluid is a clear, viscid, sterile, non-pyrogenic solution of dextran 70 (32% W/V) in dextrose (10% W/V). Dextran 70 is that fraction of dextran, a branched polysaccharide composed of glucose units, having a weight average molecular weight of 70,000. The fluid is electrolyte-free and non-conductive. At room temperature Hyskon Hysteroscopy Fluid has a viscosity of 220 cSt.

Hyskon has a tendency to crystallize when subjected to temperature variations or when stored for long periods. If flakes of dextran are present, heat at 100°-110° C until complete dissolution is achieved.

INDICATIONS: Hyskon Hysteroscopy Fluid is indicated for use with the hysteroscope as an aid in distending the uterine cavity and in irrigating and visualizing its surfaces.

CONTRAINDICATIONS: Hyskon Hysteroscopy Fluid should not be instilled in patients known to be hypersensitive to dextran. All other contraindications are those relative to the hysteroscopic procedure itself, such as pregnancy, endometrial carcinoma, etc.

WARNINGS: It is not known whether systemic absorption of dextran 70 occurs from the uterine and peritoneal cavities. Because of the possibility that absorption can occur, the physician should familiarize himself with the systemic effects of this drug. The most alarming are severe, sometimes fatal anaphylactoid reactions, which are quite rare. Other noteworthy effects include plasma volume expansion and transient prolongation of bleeding time.

ADVERSE REACTION: Undesirable effects directly attributable to the use of Hyskon Hysteroscopy Fluid have not been reported. However, the physician should be mindful of the fact that adverse reactions are known to occur with the intravenous administration of dextran 70 (MACRODEX 6% W/V). Such reactions have included: allergic phenomena (anaphylactoid reactions, urticaria, nasal congestion, wheezing, tightness of chest and mild hypotension), nausea, vomiting, fever and joint pains.

DOSAGE AND ADMINISTRATION: The amount of Hyskon Hysteroscopy Fluid required per patient depends on a number of factors, such as the type and length of the diagnostic procedure, whether or not manipulation or surgery is performed, etc. Most often, however, the amount of Hyskon instilled into the uterus will be between 50 and 100 milliliters.

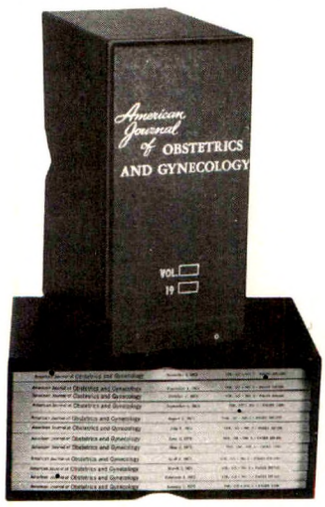
Hyskon should be introduced into the uterine cavity through the cannula of a hysteroscope under low pressure (approximately 100 mm Hg) until the uterus is sufficiently distended to permit adequate visualization. During the hysteroscopic examination, Hyskon should be infused at a rate that keeps the cavity suitably distended. To avoid injection of the fluid into the tissues of the uterus and parametria and to avoid having unnecessary amounts of the fluid pass into the peritoneal cavity and backwards along the side of the hysteroscope, infusion pressures greater than 150 mm Hg should not be used.

HOW SUPPLIED: Hyskon Hysteroscopy Fluid (32% W/V dextran 70 in 10% W/V dextrose) is available as a sterile, nonpyrogenic solution in 100 ml bottles packed 12 to a carton.
NDC No. 0016-0231-61
Keep from cold during transportation.

CAUTION: Federal law prohibits dispensing without prescription.

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this year or any year

Norlestrin® Fe 2.5 mg

Each pink tablet contains 2.5 mg norethindrone acetate, 50 mcg ethinyl estradiol. Each brown tablet contains 75 mg ferrous fumarate, USP.

Norlestrin Fe 1 mg

Each yellow tablet contains 1 mg norethindrone acetate, 50 mcg ethinyl estradiol. Each brown tablet contains 75 mg ferrous fumarate, USP.

*Both serious and minor adverse reactions have been reported by women taking oral contraceptives. The physician should be alert to their manifestations and should carefully review the following page for prescribing information.

PARKE-DAVIS

See next page for brief summary of prescribing information.

Norlestrin

(norethindrone acetate and ethinyl estradiol tablets, NF)



BRIEF SUMMARY OF PRESCRIBING INFORMATION

ACTIONS

Norlestrin achieves its contraceptive effect primarily by inhibition of ovulation through gonadotropin suppression. It is possible that other sites of action such as changes in cervical mucus and in the endometrium may contribute to the high efficacy of oral contraceptives.

SPECIAL NOTE

Oral contraceptives have been marketed in the United States since 1960. Reported pregnancy rates vary from product to product. The effectiveness of the sequential products appears to be somewhat lower than that of the combination products. Both types provide almost completely effective contraception.

An increased risk of thromboembolic disease associated with the use of hormonal contraceptives has now been shown in studies conducted in both Great Britain and the United States. Other risks, such as those of elevated blood pressure, liver disease, and reduced tolerance to carbohydrates, have not been quantitated with precision.

Long-term administration of both natural and synthetic estrogens in subprimate animal species in multiples of the human dose increases the frequency for some animal carcinomas. These data cannot be transposed directly to man. The possible carcinogenicity due to the estrogens can neither be affirmed nor refuted at this time. Close clinical surveillance of all women taking oral contraceptives must be continued.

INDICATION

Norlestrin is indicated for the control of conception.

CONTRAINDICATIONS

1. Thrombophlebitis, thromboembolic disorders, cerebral apoplexy, or a past history of these conditions.
2. Markedly impaired liver function.
3. Known or suspected carcinoma of the breast.
4. Known or suspected estrogen-dependent neoplasia.
5. Undiagnosed abnormal genital bleeding.
6. Pregnancy.

WARNINGS

1. The physician should be alert to the earliest manifestations of thrombotic disorders (thrombophlebitis, cerebrovascular disorders, pulmonary embolism, and retinal thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately.

Retrospective studies of morbidity and mortality in Great Britain and studies of morbidity in the United States have shown a statistically significant association between thrombophlebitis, pulmonary embolism, and cerebral thrombosis and embolism and the use of oral contraceptives. There have been three principal studies in Britain[1-3] leading to this conclusion, and one[4] in this country. The estimate of the relative risk of thromboembolism in the study by Vessey and Doll[3] was about sevenfold, while Sartwell and associates in the United States found a relative risk of 4.4, meaning that the users are several times as likely to undergo thromboembolic disease without evident cause as nonusers. The American study also indicated that the risk did not persist after discontinuation of administration, and that it was not enhanced by long continued administration. The American study was not designed to evaluate a difference between products. However, the study suggested that there might be an increased risk of thromboembolic disease in users of sequential products. This risk cannot be quantitated, and further studies to confirm this finding are desirable.

2. Discontinue medication pending examination if there is sudden partial or complete loss of vision, or if there is a sudden onset of proptosis, diplopia, or migraine. If examination reveals papilledema or retinal vascular lesions, medication should be withdrawn.
3. Since the safety of Norlestrin (norethindrone acetate and ethinyl estradiol) in pregnancy has not been demonstrated, it is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period.
4. A small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The long-range effect to the nursing infant cannot be determined at this time.

PRECAUTIONS

1. The pretreatment and periodic physical examinations should include special reference to breasts and pelvic organs, including Papanicolaou smear, since estrogens have been known to produce tumors, some of them malignant, in five species of subprimate animals.
2. Endocrine and possibly liver function tests may be affected by treatment with Norlestrin. Therefore, if such tests are abnormal in a patient taking Norlestrin, it is recommended that they be repeated after the drug has been withdrawn for two months.
3. Under the influence of estrogen-progestogen preparations, preexisting uterine fibromyomata may increase in size.
4. Because these agents may cause some degree of fluid retention, conditions which might be influenced by this factor, such as epilepsy, migraine, asthma, cardiac or renal dysfunction, require careful observation.
5. In breakthrough bleeding, and in all cases of irregular bleeding per vaginam, nonfunctional causes should be borne in mind. In undiagnosed bleeding per vaginam, adequate diagnostic measures are indicated.
6. Patients with a history of psychic depression should be carefully observed and the drug discontinued if the depression recurs to a serious degree.
7. Any possible influence of prolonged Norlestrin therapy on pituitary, ovarian, adrenal, hepatic, or uterine function awaits further study.
8. A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. The mechanism of this decrease is obscure. For this reason, diabetic patients should be carefully observed while receiving Norlestrin therapy.
9. The age of the patient constitutes no absolute limiting factor, although treatment with Norlestrin may mask the onset of the climacteric.
10. The pathologist should be advised of Norlestrin therapy when relevant specimens are submitted.
11. Susceptible women may experience an increase in blood pressure following administration of contraceptive steroids.

ADVERSE REACTIONS OBSERVED IN PATIENTS RECEIVING ORAL CONTRACEPTIVES

A statistically significant association has been demonstrated between use of oral contraceptives and the following serious adverse reactions:

Thrombophlebitis
Cerebral thrombosis
Pulmonary embolism

Although available evidence is suggestive of an association, such a relationship has been neither confirmed nor refuted for the following serious adverse reactions:

Neuro-ocular lesions, eg, retinal thrombosis and optic neuritis

The following adverse reactions are known to occur in patients receiving oral contraceptives:

Nausea
Vomiting
Gastrointestinal symptoms (such as abdominal cramps and bloating)
Breakthrough bleeding
Spotting
Change in menstrual flow
Amenorrhea during and after treatment
Edema
Chloasma or melasma
Breast changes: tenderness, enlargement, and secretion
Change in weight (increase or decrease)
Changes in cervical erosion and cervical secretions
Suppression of lactation when given immediately postpartum
Cholestatic jaundice
Migraine
Rash (allergic)
Rise in blood pressure in susceptible individuals
Mental depression

Although the following adverse reactions have been reported in users of oral contraceptives, an association has been neither confirmed nor refuted:

Anovulation post-treatment
Premenstrual-like syndrome
Changes in libido
Changes in appetite
Cystitis-like syndrome
Headache
Nervousness
Dizziness
Fatigue
Backache
Hirsutism
Loss of scalp hair
Erythema multiforme
Erythema nodosum
Hemorrhagic eruption
Itching

The following laboratory results may be altered by the use of oral contraceptives:

Hepatic function: increased sulfobromophthalein retention and other tests; Coagulation tests: increase in prothrombin Factors VII, VIII, IX, and X; Thyroid function: increase in PBI, and butanol-extractable protein-bound iodine and decrease in T^3 uptake values; Metyrapone test; Pregnanediol determination.

SPECIAL NOTE

In the rare case of bleeding which resembles menstruation, the patient should be advised to discontinue medication and then begin taking tablets from a new compact on the fifth day (Day 5). Persistent bleeding which is not controlled by this method indicates the need for re-examination of the patient, at which time nonfunctional causes should be borne in mind.

SD

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PARKE-DAVIS

PARKE, DAVIS & COMPANY, Detroit, Michigan 48232

PD-JA-1299-2-P (8-75)

GYNECOLOGY

Effect of oral contraceptives on rhythmic urinary metal excretion

S. WEINMANN, M.P.H.
E. B. DAWSON, PH.D.
M. H. SMOLENSKY, PH.D.
R. F. WEISBERG, M.S.
With the technical assistance of
B. LANE, B.S.
S. BRYAN, B.S.
Houston and Galveston, Texas

Thirteen women, six of whom were using oral contraceptive agents, between the ages of 23 and 34 years, free from illness, and without a previous clinical history of menstrual abnormality, were studied with chronobiological methods for circadian (about 24 hour) patterns of metal and creatinine excretion. Circadian rhythms were detected for the excretion of lithium, potassium, iron, sodium, copper, and creatinine. Statistically significant by lower 24 hour excretion (mesors) of lithium, potassium, and iron was detected for the subjects using oral contraceptive agents. Although all women adhered to nearly identical activity-rest (synchronization) schedules, the peak times (acrophases) of the metal excretory rhythms for oral contraceptive users were phase delayed by about two hours.

THE METABOLISM OF women using oral contraceptive agents is similar in many respects to that of pregnant women. In particular, the utilization of certain vitamins as well as metals seems to be affected.[1] Published works suggest oral contraceptive agents are capable of altering the serum and/or urinary levels of magnesium and potassium,[2, 3] iron,[4, 5] copper,[6–10] and zinc.[8, 9] Although the results of the earlier studies are not entirely consistent, in general, the serum levels of oral contraceptive users tend to be reduced for magnesium[2, 3] and increased for copper and iron.[6–10] Contradictory findings characterize the results of studies on zinc.[8, 9] No studies are known to these authors which pertain to the effects of oral contraceptive agents on sodium and potassium.

The methodology of studying the excretory patterns of oral contraceptive users and nonusers differed from study to study (i.e., some investigators collected blood or urine samples randomly during either the morning or afternoon, while others collected 24 hour urine specimens). Since circadian (about 24 hour rhythmic) variations characterize the serum and excretory levels of metals,[12, 27–29] a prospective study was designed with

From The University of Texas, Health Science Center, School of Public Health, Houston, and The University of Texas Medical Branch, Department of Obstetrics and Gynecology, Galveston.

Received for publication February 26, 1975.

Accepted April 21, 1975.

Reprint requests: Dr. Earl B. Dawson, Department of Obstetrics and Gynecology, The University of Texas Medical Branch, Galveston, Texas 77550.

the use of chronobiological methods. The aims of the study were to survey differences between the excretory patterns of users and nonusers of oral contraceptives and to attempt to clarify some of the reported discrepancies pertaining to the effects of oral contraceptives on metal excretion.

Methodology

Two groups of women without previous clinical history of menstrual abnormality, free of illness, and adhering to unrestricted diets were studied. Group A (control group) consisted of seven regularly menstruating women between the ages of 23 and 34 years. Group B consisted of six women between the ages of 23 and 32 years who were using a combined type of oral contraceptive—Norinyl-1 + 50 and + 80 (two subjects each),* Enovid 5 mg.† or Ortho-Novum 1/80.‡. All subjects were diurnally active. For both groups, the mean times of awakening and retiring were 07.37 and 22.55, respectively; the differences between the two groups for the times of awakening and retiring were insignificant.

Spontaneous urine samples were collected on the first day of menses and every fifth day thereafter for one complete menstrual cycle, which varied in length for the subjects from between 27 and 35 days. In the mornings on days of urine collection, each subject voided as usual. Subsequent urine samples were collected in prelabeled polyethylene bottles for the following spans: arising (about 07.30) to 12.00, 12.00 to 16.00, 16.00 to 20.00, 20.00 to bedtime (about 23.00), and bedtime to arising of the following day. Total volume was recorded for all voidings. Entire samples were frozen until analysis. In addition to collecting urine samples, each subject recorded times of arising and retiring, foods and medications consumed, and morning basal temperature measured to the nearest 0.1° F.

Laboratory analyses of urine samples were carried out with a spectrophometer.§ Modifications of standard procedures developed by Dawson[13] were used. Samples were diluted 1:51 in 0.5 per cent $LaCl_2$ solution prior to measurement of calcium, magnesium, potassium, and sodium, whereas samples were measured directly for copper, iron, lithium, and zinc. In addition, creatinine was determined by using the picric acid method of Folin and Wu.[14]

Chronobiological statistical methods, including least squares and cosinor analyses,[15, 16] were used to objectively characterize rhythms by the mesor, M (a rhythm-adjusted average), amplitude, A (equal to one half the rhythmic variability within 24 hours), and acrophase (crest time of the rhythm which refers to local midnight 00.00), along with 95 per cent confidence indices for each in the case of data documented to be circadian rhythmic. Differences between mesors were examined by Student's t test.

Results

Results of chronobiological statistical analyses are presented in Table I. Circadian rhythms were detected for both control subjects and oral contraceptive users for sodium, potassium, iron, and copper. In addition, circadian rhythms were detected for lithium and creatinine for the oral contraceptive group only. When the data of both groups were considered together, circadian rhythms were found for all the aforementioned urinary variables.

Inspection of the rhythm characteristics, especially the mesor and acrophase, reveals certain differences between the two study groups. For example, for the oral contraceptive group compared to the nonuser control group, the acrophases for all the variables occurred from two to three hours later. However, since the 95 per cent confidence intervals of the acrophases do overlap, the variation in the timing of the acrophases does not appear to be statistically significant. Nevertheless, the observation of a phase delay for the urinary rhythms of oral contraceptive users is intriguing because there is no disagreement in the sleep-activity schedule (a known synchronizer of circadian functions[30]) between the groups.

Table I also indicates differences between the mesor values of users and nonusers of oral contraceptive agents. Results of t test comparisons of mesor values also reveal statistically significant differences for urinary potassium, iron, and lithium (at the $P < 0.005$ level) and for copper (at $0.05 < P < 0.10$). For all statistically significant comparisons, higher excretion levels (mesors) characterize control subjects rather than oral contraceptive users.

Comment

In this study, chronobiological methods were used to survey urinary excretions in users and nonusers of oral contraceptives in healthy women adhering to their usual diet. Circadian rhythm was documented for

*Syntex Laboratories, Inc., Stanford Industrial Park, Palo Alto, California 94304.

†G. D. Searle & Co., P. O. Box 5110, Chicago, Illinois 60680.

‡Ortho Pharm. Corp., Route 202, Raritan, New Jersey 08869.

§303 Atomic absorption spectrophotometer, Perkin-Elmer Corp., Instrument Div., 800 Main Ave., Norwalk, Connecticut 06852.

Table I. Circadian urinary rhythms for six oral contraceptive agent users and seven control subjects

Urine variable	*Group Composition*	*Mesor* (Mean ± S.E.)*	*Amplitude† (95% confidence limits)*	*Acrophase‡ (95% confidence limits)*	*P value by t test§*
Sodium	Control‖	0.995 ± 0.080	0.229 (0.095 to 0.363)	15.04 (11.08 to 17.32)	0.2
(mmoles × 51/L./hr.)	User‖	1.139 ± 0.083	0.317 (0.128 to 0.506)	17.56 (14.32 to 20.52)	<P<
	Both‖	1.060 ± 0.006	0.251 (0.120 to 0.382)	16.40 (14.08 to 18.56)	0.4
Potassium	Control‖	1.303 ± 0.125	0.681 (0.348 to 1.014)	12.16 (11.40 to 15.04)	0.001
(μmoles × 51/L./hr.)	User‖	0.884 ± 0.066	0.396 (0.237 to 0.555)	14.08 (12.40 to 17.24)	<P<
	Both‖	1.110 ± 0.080	0.539 (0.413 to 0.665)	13.04 (12.16 to 14.52)	0.005
Iron	Control¶	0.137 ± 0.010	0.032 (0.008 to 0.057)	14.24 (08.44 to 16.52)	0.001
(μmoles/L./hr.)	User#	0.102 ± 0.007	0.025 (0.009 to 0.041)	16.24 (12.44 to 20.12)	<P<
	Both#	0.121 ± 0.006	0.228 (0.009 to 0.046)	15.12 (12.16 to 17.40)	0.005
Copper	Control¶	0.045 ± 0.006	0.017 (0.006 to 0.028)	12.04 (10.20 to 16.04)	0.05
(μmoles/L./hr.)	User¶	0.032 ± 0.005	0.013 (0.003 to 0.023)	14.08 (12.24 to 22.52)	<P<
	Both#	0.039 ± 0.004	0.014 (0.004 to 0.024)	12.52 (11.40 to 16.16)	0.1
Lithium**	Control††	0.456 ± 0.052	0.008	17.28	0.001
(μmoles/L./hr.)	User#	0.271 ± 0.220	0.074 (0.017 to 0.131)	20.00 (12.24 to 19.40)	<P<
	Both#	0.374 ± 0.031	0.077 (0.020 to 0.134)	18.32 (15.04 to 21.20)	0.005
Creatinine**	Control††	32.424 ± 1.872	3.224	16.36	0.2
(mg./100 ml.)	User#	35.497 ± 1.229	5.510 (1.990 to 9.030	15.56 (12.24 to 19.40)	<P<
	Both‖	33.853 ± 1.165	4.272 (1.870 to 6.670)	16.12 (12.40 to 19.44)	0.4

*Mesor = the rhythm-adjusted 24 hour mean.

†Amplitude = ½ of the rhythmic variation within 24 hours.

‡Acrophase = the crest of the rhythm, approximated by curve fitting; Ø given in hours and minutes and referenced to local 00.00.

§Tests of mesor values for control group and oral contraceptive users.

‖$P < 0.001$.

¶$P < 0.05$.

#$P < 0.01$.

**The 95 per cent confidence limits for amplitude and acrophase are given only when rhythm detection was significant at $P < 0.05$.

††Not significant.

several urinary metals. The detection, as well as the rhythm characteristics (M, A, and Ø),[11, 17–21, 27–29] of the circadian patterns is in agreement with results shown in the literature. Although the results suggest the existence of a slight phase delay in the urinary rhythms of oral contraceptive users, of more importance is the documentation of differences in the urinary excretion of certain metals in users as compared to nonusers.

While some studies document oral contraceptive effects upon the serum levels of magnesium, calcium, iron, copper, and zinc,[1–3, 6–10] only a few studies are available for comparing the urinary patterns of users and nonusers. Nonetheless, differences between the circadian mesors for the variables of potassium, iron, and lithium reported herein for urine are for the most part consistent with findings previously published. For example, the results of earlier studies[4, 5] document that the dietary requirement of iron in women using oral contraceptives may be slightly lower than that of nonusers, perhaps due to a reduction in the duration and quantity of menstrual bleeding. Goldsmith and associates[2] reported differences, although nonsignificant ones, in the urinary and serum magnesium levels of OCA users as compared to nonusers. Earlier works also indicated serum copper levels to be slightly increased in oral contraceptive users.[6, 8–10] Studies on rats show that estrogen can decrease the plasma zinc level and affect its metabolism in the liver, spleen, adrenal, and uterus.[1]

The findings of differences between the two groups for the urinary concentrations of potassium, iron, lithium ($P < 0.005$), and copper at ($0.05 < P < .10$) must be evaluated with caution. First, the results from this initial survey may reflect dissimilar dietary habits of the two groups. Although inspection of the dietary records kept by the subjects indicates no significant differences between the groups for food intake, detailed studies with controlled diets will be required to verify the findings reported herein. Second, our results document differences in the urinary excretion of the aforementioned metals; they do not in themselves represent changed requirements in the mineral requirements of users as compared to nonusers. Third, one of the assumptions underlying the design of this study is that the circadian mesors (for the control group and the oral contraceptive users prior to commencement of oral contraceptives) for metal excretion was nearly identical. Certainly, this assump-

tion is tenuous since a great amount of data suggest that there are individual variations in physiologic regulation and, indeed, autorhythmometry (self-measurements taken several times daily for many days on meaningful biological indicators, such as blood pressure, pulse, and peak expiratory flow rate[22–23] documents such.

Based upon the experiences of this survey, it is suggested that future studies be carried out on one group of subjects, first during the span of one or more menstrual cycles prior to oral contraceptive medication and then for an identical span of time during which oral contraceptives are taken. Furthermore, during such a study, it seems mandatory, in order to obtain a representative picture of metabolic events both before and during oral contraceptive use, that control diets be implemented. For both blood and urine sampling, because circadian and possible menstrual rhythms are known for a great number of physiologic and biochemical functions,[24–26] it is required that chronobiological methodologies be used. This includes the standardization of subjects to a common sleep-activity schedule, the use of appropriate sampling methodologies (such as the collection of data at regular intervals during designated 24 hour spans scheduled at least at seven day intervals throughout one or more menstrual cycles), and the utilization of chronobiological statistical techniques to objectively quantify data collected over time.

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An investigation of the effects of the intrauterine contraceptive device based on a longitudinal study of a self-selected sample of Barbadian women

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The results of a longitudinal study of a self-selected sample of 1,790 Barbadian women who accepted the intrauterine contraceptive device (IUD) as their method of contraception are reported. The accumulated experience of 44,000 woman months of IUD use is presented. The demographic, medical, and obstetric data on admission for 2,797 IUD acceptors and 4,296 nonacceptors are contrasted. The reproductive experience of the two groups prior to the insertion of an IUD with respect to live births, stillbirths, and miscarriages is similar while the incidence of ectopic pregnancy is shown to be different. The association between the IUD and the incidence of tubal pregnancy is evaluated, and the literature dealing with this topic is reviewed. Estimates of the relative risk of an ectopic pregnancy in a woman with an IUD in utero are given.

IN THIS PAPER, the results obtained in a study of a self-selected sample of 2,797 women in the reproductive years from Barbados, West Indies, who accepted and used an intrauterine contraceptive device (IUD) are reported. The demographic characteristics and past obstetric histories of the group of IUD acceptors are discussed and contrasted with those of 4,296 Barbadian women who did not choose to have an IUD. The experience observed in a prospective longitudinal study of 1,340 IUD wearers with an aggregate of 44,790 woman months of use is presented. The data reported by other investigators concerning the relative risk of ectopic pregnancy in women who become pregnant with an IUD in situ are reviewed, and the findings of this study are examined.

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Table I. Distribution of IUD acceptors and nonacceptors by age

Age (years)	*IUD acceptors*	*Non acceptors of IUD*	*Total*
15-19	138	562	700
20-24	779	1,189	1,964
25-29	657	651	1,308
30-34	541	627	1,168
35-39	440	737	1,177
40-44	242	530	772
Total	2,797	4,296	7,093

Methods and procedures

The details of the design and the procedures employed in the study from which the data reported here were obtained have been discussed in previous reports[1, 2]. Briefly, a family planning–Papanicolaou smear program was undertaken in Barbados, West Indies, in 1966. During the following six years, open clinics were established and maintained throughout the island of Barbados. Active recruitment of women in the childbearing ages was carried out. The IUD was promoted as the method of choice for this family planning program. However, patients were free to choose any method of contraception. IUD insertion and all other services of this program were provided

Table II. Distribution of IUD acceptors and nonacceptors by number of live births, stillbirths, and miscarriages

	Live births		*Stillbirths*		*Miscarriages*	
No.	*IUD acceptors*	*Nonacceptors of IUD*	*IUD acceptors*	*Nonacceptors of IUD*	*IUD acceptors*	*Nonacceptors of IUD*
0	53	923	2,513	3,962	2,098	3,397
1	314	771	112	149	377	522
2	413	624	19	21	123	151
3	420	470	7	8	37	54
4	383	381	2*	7*	18*	24*
5	288	332				
6	286	229				
7	213	153				
8	150	117				
9	106	105				
≥10	171	191				
Not recorded	—	—	144	149	144	148

*≥4.

Table III. Distribution of IUD acceptors and nonacceptors according to number of ectopic pregnancies

No. of ectopic Pregnancies	*IUD acceptors*	*Nonacceptors*	*Totals*
0	2,632	4,103	6,735
1	19	44	63
Not recorded	146	149	295
Total	2,797	4,296	7,093

without charge. Upon admission to the clinic, the patient had a detailed interview dealing with past medical, gynecologic, and reproductive history and a physical examination. At the time of the gynecologic examination, a Papanicolaou smear was taken, and, if an IUD was desired, it was inserted then. A return visit was set up at the time of the initial visit according to a specific schedule that varied with intervals ranging from three months to one year. The strategy for any missed follow-up visits included repeated mailings of a request to return for the missed visit followed by a home visit from a visiting nurse.

Admission data

The age distribution of the IUD users and women who did not accept the IUD is shown in Table I. There are some differences in the age distribution of the two groups. There is a comparative underrepresentation (4.93 per cent) in the age group less than 20 years old among the IUD acceptors when contrasted with the proportion in this age group who did not use the IUD (13.08 per cent); and there is a lower proportion of IUD users in the age group over 40 years old. A higher proportion of IUD acceptors are in the age group 20 to 34 years old when contrasted with that noted among the nonacceptors. These differences are concordant with those that would be expected given that this is a self-selected sample—where selection is based, in part, on parity and marital status. These factors can be shown to be correlated to the observed differences in this sample.

The reproductive history of the two groups is summarized in Tables II and III. The contrasts shown represent the two subsets in the sample—IUD acceptors and nonacceptors—at the time of the initial visit. In each group contraception was practiced by approximately 60 per cent of women in the sample using those means available prior to the initiation of this study. These include the condom, foam, oral contraceptives, and the diaphragm. Contrasts of the two groups with respect to prior contraceptive measures used were unremarkable.

The distribution of the two groups, acceptors and nonacceptors, by number of live births, stillbirths, and miscarriages is shown in Table II. It is of interest to note that 53 nulliparas accepted the IUD as their method of choice for contraception. The highest proportion of IUD acceptors (58 per cent) was found among women who had seven live births. Over 50 per cent of women in this sample with four or more live births accepted the IUD. There are no differences in the experience of these two groups with respect to the observed frequency of stillbirths. Less than 1 per cent of the women in each group had more than one stillbirth. Approximately 90 per cent of the women in each group had no prior stillborn children. While some question may be raised about the reliability of these data, the experience observed in this sample is not significantly different from that reported by other

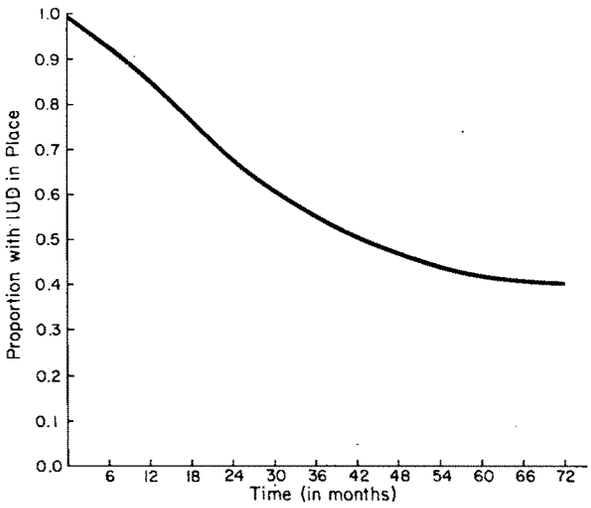


Fig. 1. IUD usage over time among Barbadian women.

investigators who report data based on information obtained by patient interview. There are no significant differences between the two groups with respect to the frequency of miscarriages. It is of interest to note that in both groups approximately 2 per cent of women experienced three or more miscarriages. There is a high reported incidence of fetal wastage in this sample. Although some question remains concerning the reliability of the information obtained at the initial interview, there is a high proportion of grand multiparas in this sample, and other appropriate data bases are not available which are suitable for contrast with the experience in Barbadian women reported here.

The frequency distribution of ectopic pregnancies in the two groups is shown in Table III. It is difficult to evaluate or contrast these data with the results of other investigators. Although there is no statistically significant difference between the two groups, there is a slightly lower incidence of ectopic gestations noted among those women who accepted the IUD when the data are analyzed as they are presented in Table III. There are a total of 13,708 and 14,536 completed pregnancies among the samples of acceptors and nonacceptors, respectively. Based on these data, the ectopic pregnancy rate in this sample is 0.139 and 0.303 per 100 fertilized ova for the two cohorts, that is, the rate of ectopic pregnancy among the IUD acceptors is less than one half that of the nonacceptors. When the data are viewed in this manner, with the number of ectopic pregnancies measured as a function of the total number of gestations, there is a statistically significant difference between the two groups prior to the insertion of the IUD.

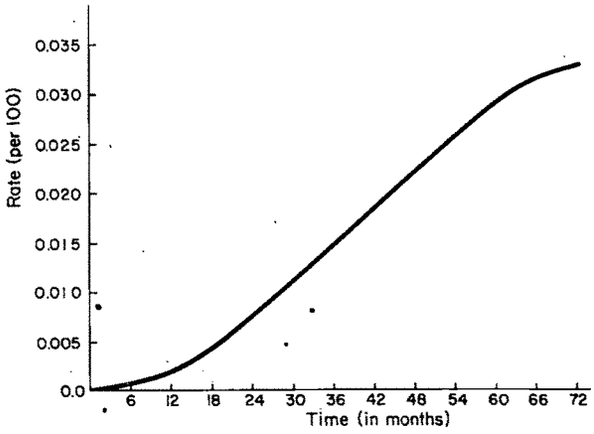


Fig. 2A. Gynecologic surgery while loop was in place.

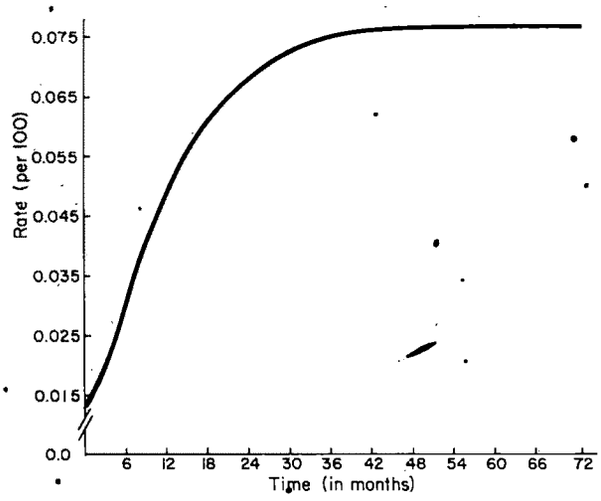


Fig. 2B. Incidence of loop expulsion (reinserted > 1 day later).

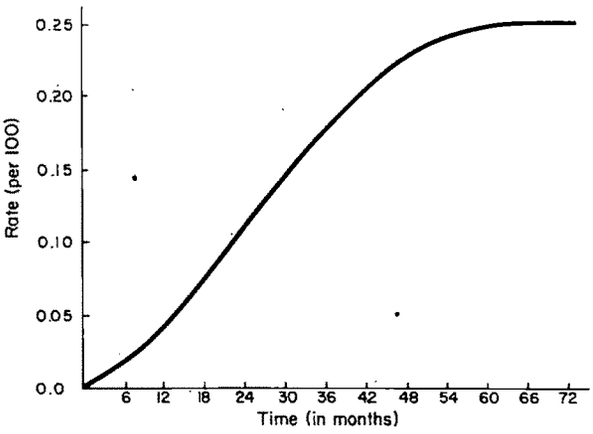


Fig. 2C. Loop removals for medical and nonmedical reasons.

Table IV. Cumulative IUD use and pregnancy in cohort of 1,390 women

Ordinal month	*Porportion of women with continuous follow-up*	*Cumulative No. of months of IUD use*	*Cumulative No. of pregnancies*
1	0.992	1,384.5	0
2	0.978	2,753.5	0
3	0.965	4,099.0	0
4	0.954	5,423.0	1
5	0.944	6,731.5	2
6	0.934	8,024.5	3
7	0.918	9,297.0	4
8	0.907	10,549.5	5
9	0.891	11,783.0	8*
10	0.883	12,998.5	8
11	0.871	14,198.5	8
12	0.864	15,383.5	8
13	0.831	16,521.0	9
14	0.813	17,590.5	11
15	0.800	18,617.0	12
16	0.788	19,611.0	13
23	0.691	25,808.0	14
32	0.597	31,885.5	15*
38	0.537	35,033.0	16

*Addition includes one ectopic pregnancy.

Other variables which have been examined for the two subsets—acceptors and nonacceptors—include: (1) age at first intercourse, (2) religion, (3) educational status, (4) number of sexual partners, and (5) prior history of pelvic inflammatory disease or gynecologic surgery. In no instance were there any significant differences between these two cohorts with respect to these variables.

Longitudinal experience

The longitudinal experience observed in 1,390 women who used the IUD (Lippes loop C and D) in the age group 20 to 39 years old is shown in Figs. 1 to 2C. For this subset, at least one follow-up clinic visit was made. The relevant demographic characteristics, i.e., age, parity, etc., of this sample do not differ from those of the entire group of IUD acceptors.

The longitudinal experience of women who accepted the IUD is shown in Fig. 1. At the end of six years of follow-up, approximately 40 per cent of women in whom an IUD had been placed continued to use this method of contraception.

The termination of IUD use due to gynecologic surgery, loop expulsion, and loop removal for medical and nonmedical reasons is presented in Figs. 2A to 2C. A total of 29 women with IUD's had gynecologic surgery performed during the six-year follow-up period. The distribution is essentially linear with a small slope to the line. There is no suggestion that the experience in this group is different from that observed among those who did not have an IUD inserted.

The spontaneous expulsion rate noted in this sample is described by a hyperbole with a plateau at approximately 8 per cent. The longitudinal experience in this cohort is not different from that reported by other workers.[3]

The longitudinal experience in the sample of IUD removals for medical and nonmedical reasons is also presented in Fig. 2. The cumulative asymptote in this instance is approximately 25 per cent. While there is concordance between these results for the study reported here and that presented by the Cooperative Statistical Program,[3] the rates of loop removal during the first and second years for the Barbadian women are approximately one half those reported in the Cooperative Study. It should be noted that the medical reason noted for removal of the IUD was clinical evidence of pelvic inflammatory disease in approximately 75 per cent of those women for whom the removal was for medical cause.

In this cohort of 1,390 women, 16 women became pregnant with an IUD in place. Two of these pregnancies, one at nine months and one at 32 months after IUD insertion, were ectopic gestations. The cumulative incidence of pregnancy among IUD acceptors in this sample is shown in Table IV. A review of the findings of other investigators is of use in order to obtain a clinically meaningful estimate of the relative risk of ectopic pregnancy in a women with an IUD in situ. Grafenberg[4] was apparently the first investigator to appreciate an increased risk of extrauterine pregnancy among IUD wearers. In a study of 1,713 patients with a cumulative experience of 21,909 woman months, Lippes[5] reported that 23 patients became pregnant with a loop in situ. Four of these women were noted to have tubal ectopic gestations, resulting in a rate of 17.4 per 100 pregnancies. A slightly lower incidence rate, 16.7/100, of ectopic pregnancy (three ectopic gestations in a total of 18 pregnancies) among women with a Margulies spiral in situ was noted by Willson and associates[6] in a study including 623 women followed for 5,606 woman months. In a follow-up study in 1968, Willson and Ledger[7] reported that among 710 women with an IUD in place followed for an aggregate of 14,901 woman months 29 pregnancies were reported. Of these, five were noted to be tubal ectopic pregnancies, resulting in a rate of 17.2/100 pregnancies. In similar studies, Hall[8] reported that of 76 pregnancies among women with an IUD in place three were noted to have ectopic implantations (3.9/100). Wei[9] reviewed the data per-

taining to ectopic pregnancy among women in Taiwan who used IUD's. He showed that from 1964 to 1967 the number of IUD users in his sample increased and simultaneously there was an increase in the number of ectopic gestations among this group. However, the total number of ectopic pregnancies remained unchanged in the non–IUD users. He further showed that the per cent of ectopic pregnancies among IUD users ranged from 8.3 to 20.6 per cent of the total number of gestations while the per cent of ectopic pregnancies in the population ranged from 3.4 to 4.3 per cent during this same period.

More recently, Tietze[10] reviewed the incidence of ectopic pregnancy in a large sample of IUD users. In 783 women who became pregnant with an IUD in situ, 34 pregnancies were found to be ectopic (4.3/100) pregnancies). He then developed a model concerning the association between the IUD and the incidence of ectopic pregnancy. For a noncontracepting sample the assumptions are: (1) there is a constant probability of fertilization of ova of from 20 to 30 per 100 woman months, therefore, with 13 ovulations per year, the annual rate of fertilization would be between 260 and 390 per 100 women; (2) the incidence of ectopic pregnancy is approximately 0.3 per 100 fertilized ova. With these, it can be shown that between 0.8 and 1.2 ectopic pregnancies per 100 woman years can be expected. Tietze then showed, based on these estimates, that in the large sample being studied between 229 and 343 ectopic pregnancies could be anticipated in the entire cohort of women included in that study. In fact, only 34 ectopic pregnancies were noted. In a subsequent report using these same assumptions and a more extensive data base, Lehfeldt and colleagues[11] suggested that although the incidence of ectopic pregnancy is very high in relation to the total number of pregnancies it is very low in relation to the calculated expected number. This finding supported the conclusion that the IUD interferes with conception in the Fallopian tubes as well as the uterus. He suggested that the IUD reduced uterine implantation by about 99.5 per cent, tubal implantation by 95 per cent, and the incidence of ovarian pregnancy not at all. It should be noted here that Tietze's calculations result in a smaller decrement in the incidence of ectopic pregnancy than in that noted for intrauterine gestation. This finding is in agreement with the noted increased relative risk of ectopic pregnancy among a cohort of IUD wearers.

It is difficult to interpret contrasts between the sample under study here and those of other investigations. The reported rates of ectopic pregnancies among IUD users range from 3.9 to 17.2/100 pregnancies. In any event, the question of clinical interest is that if a woman has an IUD in place *and* is pregnant what is the probability that she has an ectopic pregnancy relative to that probability for a pregnant woman who is not using an IUD? Using the estimated number of fertilized ova/100 woman years and the estimated rate of ectopic gestations in a noncontracepting population presented by Lehfeldt and colleagues, [11] it can be shown that the relative risk of an ectopic pregnancy ranges from 0.002 to 0.004 and the relative risk for a cohort of IUD users ranges from 0.01 to 0.02, that is, there is a 2.5-fold to a tenfold increase in the relative risk of ectopic pregnancy in an IUD wearer contrasted to that in a woman without an IUD in utero. In fact, when the relative incidence of ectopic pregnancy among IUD users reported by other investigators is reviewed, it appears that the *lower* bound for the difference between these two probabilities is in the range of a factor of 10, that is, the probability of a tubal pregnancy among IUD wearers who become pregnant is at least 10 times greater than it is for a woman who becomes pregnant without an IUD in situ. Indeed, the relative risk may be as high as 50 times that noted among women who do not have an IUD.

In our data the incidence of ectopic pregnancy among the two self-selected groups prior to admission to this study was in the range of 0.3/100 pregnancies among those who did not accept the IUD. This is consistent with the estimate calculated by Tietze and is a part of the model he proposed. The comparable figure for the cohort of women who accepted the IUD in our study was 0.139, that is, prior to having an IUD inserted, this group had a cumulative experience of ectopic pregnancy of less than one half that noted for the contrast group. The consistent increase in this base rate among IUD wearers of the order of magnitude that has been observed would suggest that the hypothesized effect of the IUD of lowering the incidence of tubal pregnancies based on the assumptions proposed by Tietze may be an inappropriate formulation. The women who accepts an IUD as the method of contraception is in many ways selected. The criteria of selection are, in fact, associated with the incidence of a prior ectopic tubal pregnancy. This factor and others which affect the selection process may indeed result in substantial modification of the assumptions which underly the suggestion that the IUD decreases the incidence of ectopic pregnancy. In any event, the clinically meaningful question remains: "What is the probability of a tubal pregnancy given a woman with an IUD becomes pregnant?" The appropriate denominator for evaluating this question is pregnancies, not ovulations, and it is to the question posed above that the clinician must respond.

Table V. Distribution of women with positive Papanicolaou smears at the time of IUD insertion

Smear diagnosis	*No. of women*
3	52
3A	9
3B	22
3C	10
4	6
5	1
Total	100

Table VI. Distribution of women whose smear diagnosis at insertion of IUD was 2R and whose next smear was positive

		Time in months between first and second smear	
Smear diagnosis	*No. of women*	*Mean*	*Range*
3	6	9.8	1-26
3A	1	2	—
3B	2	3	1 and 5
3C	1	14	—
4	2	4	1 and 7
Total	12		

Table VII. Distribution of women with no smear at insertion of IUD whose first smear was positive

		Time in months between insertion of IUD and first Papanicolaou smear	
Smear diagnosis	*No. of women*	*Mean*	*Range*
3	21	13.5	4-43*
3A	5	20	12-29*
3B	7	11.4	1-25*
3C	1	17	—
4	1	11	—
Total	35		

*Range between time of insertion and follow-up examination with Papanicolaou smear.

Loop insertion—intraepithelial neoplastic disease

The design of the family planning program from which these data were generated resulted in the insertion of an IUD at the patient's request at the time of her first clinic visit; at that time, a Papanicolaou smear was also taken.

The classification of the Papanicolaou smear used here has been described elsewhere.[1] Briefly, the smear classification is as follows: Class 1, normal (normal cells); Class 2R, repeat (insufficient for diagnosis); Class 3, very mild dysplasia; Class 3A, mild dysplasia; Class 3B, moderate dysplasia; Class 3C, severe dysplasia; Class 4, carcinoma in situ; Class 5, invasive carcinoma.

As a result of the design of the family planning program, 100 women who had IUD's inserted at the time of the initial visit had Papanicolaou smears taken also at the same time which were subsequently found to be positive. The smear classification for these women is shown in Table V. Thirty-nine women had the IUD inserted and were found, based on the Papanicolaou smear taken at that time, to be in smear Classes 3B, 3C, 4, or 5. Although one would not usually insert an IUD in women with severe dysplasia or carcinoma in situ, the logistics of this project resulted in insertion at the time the initial smear was taken. In Table VI, the smear diagnosis obtained at the time of the first follow-up visit for women who had a loop inserted and for whom the initial Papanicolaou smear was inadequate for diagnosis is shown. The average time between the two clinic visits is also presented. Again, the five women with smear diagnoses of 3B, 3C, and 4 are of concern in this context. Finally, 35 women in the study had no Papanicolaou smear at the time of insertion of the Lippes loop, and at their first follow-up visit these women had a Papanicolaou smear taken that was read as positive. These results are shown in Table VII. With the procedures outlined above, 53 women had IUD's inserted in the presence of abnormal Papanicolaou smears (Classes 3B, 3C, 4, and 5).

We have discussed the distribution of progression rates from dysplasia to carcinoma in situ in previous reports.[12, 13] Although we have shown that the IUD does not exert a carcinogenic effect, in view of the natural history of cervical intraepithelial neoplastic disease, we believe that those women with positive Papanicolaou smears should be treated prior to loop insertion. In this study the IUD was removed at the time of the first follow-up visit in all women whose initial Papanicolaou smear was other than Class 1.

Conclusions

1. The selection of the IUD as the contraceptive of choice is associated with age, parity, and prior history of ectopic pregnancy.

2. There are no differences between women who choose the IUD and those who do not with respect to prior miscarriages or stillbirths.

3. There is a statistically significant difference between the two groups studied, IUD acceptors and nonacceptors, with respect to the incidence of ectopic pregnancy prior to the insertion of the IUD.

4. The difference in the base incidence rate of

ectopic pregnancy may be an important factor in interpreting the effects of the IUD on the incidence of tubal gestation.

5. The incidence of ectopic pregnancy among women with IUD's in utero who become pregnant is from 10 to 50 times that noted among women who do not have an IUD in situ. This finding is true in the aggregate and in the individual.

6. Approximately one half of the 1,390 women who accepted the IUD as the contraception of choice still had the device in place three and a half years after insertion.

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Experience in mass Papanicolaou screening and cytologic observations of teen-age girls

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This report is based on a seven-year retrospective analysis of computerized data available from the Chicago Board of Health's Cancer Control Section and its Cytology Laboratory. All patients included were medically indigent. The cytologic specimens were obtained by the VCE technique; all Papanicolaou smears were classified with both the numerical and descriptive systems for abnormal cytology. The study cohort was composed of 33,641 teen-age patients through age 19; 58 (1.7/1,000) had abnormal cytology (Class III, IV, or V). In Chicago, from 1962 through 1969, approximately 25 per cent of the total patient load were teen-agers under the age of 19 with an extremely low yield of suspect cytology: Class III = 1.6 per month; Class IV = 0.1 per month; Class V = 0.0 per month. The Chicago Board of Health has reduced the number of Papanicolaou smears taken on young women under the age of 19; however, this conceptual approach does not exclude women under age 19 who epidemiologically and/or clinically warrant the implementation of a Papanicolaou smear with the use of Friedell's "biologic age of the cervix" formula.

INVASIVE CERVICAL carcinoma in the teen-age girl is a rare occurrence. Pollack and Taylor[8] stated that despite the occasional occurrence of uterine cancer in young women its presence in those below 20 years of age must be classified as exceedingly rare. Speert[7] said that, "So rare is carcinoma of the cervix in minors that such cases constitute gynecologic curiosities." Bowing and McCullough,[6] in reviewing 3,000 invasive carcinomas of the cervix at the Mayo Clinic, found one patient under 20 years of age. Additionally, they reviewed the literature and found only 25 cases since 1862, of carcinoma of the cervix in girls 20 years of age or younger. Koblanck reviewed 6,354 cases of invasive carcinoma of the cervix and found two cases in patients under 20 years of age. Huffman,[9] in his textbook *The Gynecology of Childhood and Adolescence,* stated that cervical carcinoma rarely occurs in girls under 17 years old, and, in several thousand patients examined at Children's Memorial Hospital and in private offices, no abnormal cytology was found. Yet he recommends that vaginal cytologic smears should be taken and examined for tumor cells in all adolescents, especially those who are believed to have had coitus. He also reviewed the literature since 1888, and found 32 authentic reports of cervical carcinoma in teen-agers.

Despite the rarity of the disease in teen-agers, with the establishment of cytology as an important diagnostic tool, reports began to appear in the literature on cytology in the teen-ager. Papers reflecting extreme divergence of experience have been published.

Ferguson[1] reported that his experience strongly supported the opinion that teen-age female patients should not be denied a cancer smear during a routine pelvic examination. Of 77 positive cytologic smears in girls under 20 years old (a projected rate of 30 per thousand) 10 patients had carcinoma in situ. No cases of invasive carcinoma was found. He concluded that if a girl is old enough to have a vaginal examination she is old enough to have a cervical cytologic examination.

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Table I. Cytology by classes for group ≤ 19 years old, from the Cancer Control Program, Division of Adult Health and Aging. This retrospective analysis is from January 1, 1962, to May 31, 1969.

Class	*Total white patients*	*No.*	*Total black patients*	*No.*	*Cumulative*	*Rate**	*Rate†*
0	187	49	1,386	46	1,573	45	47
1	3,532	925	27,111	909	30,643	868	911
2	92	24	1,275	43	1,367	39	41
3	6	1.6	48	1.6	54	1.5	1.6
4	–	–	4	0.1	4	0.1	0.1
5	–	–	–	–	–	–	–
Race and age unknown	–	–	–	–	1,651	47	–
Total	3,817		29,824		35,292* (33,641†)		

*Including race and age unknown.
†Excluding race and age unknown.

Christopherson,[3] in 1965, stated that the prevalence of positive cancer smears in teen-age girls examined in his clinic differed extremely from that in Ferguson's report, and he believed it was important to record this conflicting experience and place the relative risk in its proper perspective. In a study of 5,061 girls 19 years of age or less, the results of cell studies revealed neither carcinoma in situ nor invasive carcinoma. A rate of significantly abnormal findings of 1.2 per 1,000 in teen-age girls was observed. In addition, Christopherson quoted Fidler's experience showing a single carcinoma in situ and no invasive carcinoma from 7,175 teen-age girls in British Columbia. Christopherson concluded that, "Screening for cervical cancer in teen-agers is not a very rewarding endeavor even in the lower socioeconomic segment of the population. It is important to identify the high risk groups and give priority to effort where it will do most good. Until more data are accumulated, we shall continue to screen all women for cervical cancer regardless of age. At the same time, we will continue to suggest that one should concentrate on the high risk groups with teen-age girls having a very low priority."

In two papers (1965 and 1970), Kaufman and associates[2, 5] reported their observations of 10,246 teen-age patients. In this group there were 296 inconclusive and six positive smears. Three hysterectomies were performed for reasons other than anticipated cervical disease. Their statistics revealed one carcinoma in situ per 2,000 women screened and one per 1,000 with severe dysplasia. Fifty-eight patients were found to have changes suggestive of either severe dysplasia or carcinoma in situ. This represents 5.6 cases per 1,000 girls screened. When 175 patients with a cytologic diagnosis of moderate dysplasia were included, 23 patients per 1,000 screened were found to have some degree of significant cervical atypia. They concluded that their findings strongly confirm the advisability of obtaining cervical-vaginal smears on all gynecologic and obstetric patients regardless of age. This is especially true in the parous and nonvirginal patient.

Friedell,[4] commenting on the need for routinely examining smears from teen-age girls as implied by the report of Ferguson, stated, "Perhaps the best solution would be to consider the biologic age of the cervix in the individual girl, and not the chronologic age of the girl."

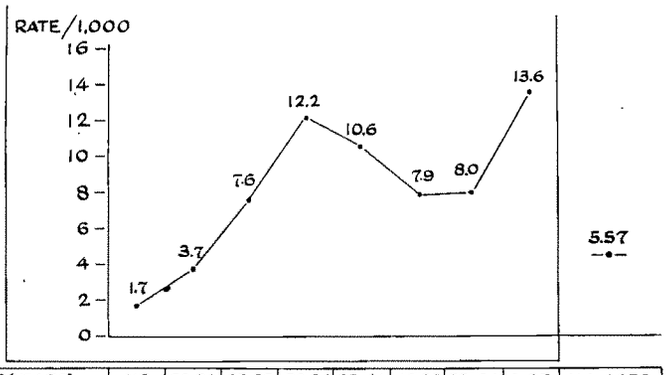
He further stated, "that perhaps we might simplify the decision-making process for the clinician by using the following equation with K_F as the constant:

$$\text{Biologic Age of Cervix} = \text{Age of Girl} + K_F \text{ (number of years of active sex life prior to age 20)}$$

In this equation Biologic Age of Cervix = Age of Girl plus number of years of active sex life before reaching age 20.

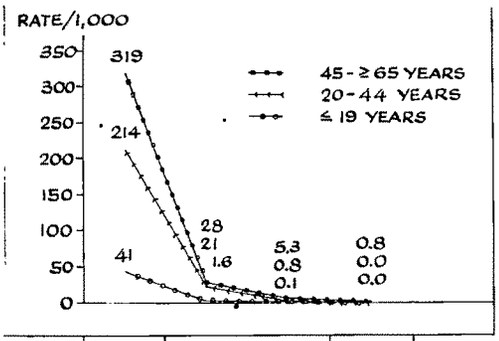
This equation is derived by assuming that an active sex life is associated with the development of cervical cancer, and that an average of five (5) years will elapse between the onset of this type of life and the onset of invasive cervical cancer. From available data this five (5) year period would seem to be a rather conservative estimate, and ten (10) years would probably come closer to the truth. Therefore when $K_F = 5$ or more, a smear must be taken. When K_F = less than 5, a smear need not be taken. With this formula it seems likely that cervical smears would be required from a relatively small number of teen-age girls."

The ongoing controversy, as evidenced by the aforestated scientific literature excerpts, established the need and set the background for a retrospective analysis of the cytology studies accomplished by the Cancer Control Section of the Chicago Board of Health.



AGE GROUP (YEARS)	≤19	20-24	25-29	30-34	35-44	45-55	55-64	≥65	ALL AGES
NO. OF OCCURRENCES	58	150	172	163	115	21	15	11	705
TOTAL OBSERVATIONS	33,641	41,059	22,356	13,817	10,863	2,653	1,865	812	126,566

Fig. 1. Rate per 1,000 of Classes *3*, *4*, and *5* Papanicolaou smears combined, for groups ≤ 19 to ≥ 65 years old, from the Cancer Control Program, Division of Adult Health and Aging. This retrospective analysis is from January 1, 1962, to May 31, 1969.



AGE GROUP (YEARS)	CLASS 2	CLASS 3	CLASS 4	CLASS 5	TOTAL OBSERVATIONS
45-≥65	491	34	13	0	5,330
20-44	4,762	515	80	5	87,595
≤19	1,367	54	4	0	33,641
	NUMBER OF OCCURRENCES				

Fig. 2. Rate per 1,000 of Classes *2*, *3*, *4*, and *5* Papanicolaou smears, for groups ≤ 19, 20 to 44, and 45 to ≥ 65 years old, from the Cancer Control Program, Division of Adult Health and Aging. This retrospective analysis is from January 1, 1962, to May 31, 1969.

Methods and material

The specimens were obtained by the VCE technique. These smears were obtained from patients attending the Chicago Board of Health Clinics and consisted of Prenatal Clinics, Family Planning Clinics, Model Cities and Federally sponsored Community Health Centers, and other programs.

Patients whose cytologic evaluations were found to be abnormal, which in our category included Classes III, IV, and V, were instructed to have: (1) A repeat Papanicolaou smear, (2) treatment and repeat Papanicolaou smear, and (3) referral for a histologic verification.

Classification of smears. A variety of authors have written about the desirability of dropping the old numerical classification system and designating all smears descriptively. In practice, however, it is quite difficult to undo the familiarity of working for two decades with a numerical system. Therefore, we have routinely used both the numerical and descriptive systems to classify abnormal smears. The numerical system is the basis for computer input. Since the onset of the program, we have obtained data regarding treatment and follow-up of patients with abnormal cytology.

Results and follow-up

This report is based on a retrospective analysis of computerized data available from January 1, 1962, through May 31, 1969. All patients included in this

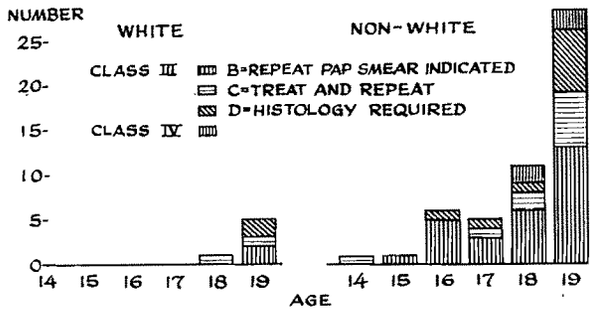


Fig. 3. Cytology distribution of 58 patients with abnormal smears by class, age, and race, from January 1, 1962, to May 31, 1969.

study were considered to be medically indigent, and the population screened originated from the more densely populated and underprivileged socioeconomic clusters within the city of Chicago. The study cohort was composed of 33,641 teen-age patients through age 19; 3,817 were white, and 29,824 were nonwhite. Of this group, 58 patients had abnormal cytology; six white patients had Class III smears, a rate of 1.6 per thousand. Forty-eight nonwhite patients had Class III smears, a rate of 1.6 per thousand. It is interesting to note that the rate per thousand is identical for this age group. The cumulative total Class III smears for white and nonwhite patients was 54, a rate of 1.6 per thousand. Four nonwhite patients were included within the Class IV category, or 0.1 per thousand. There were no Class V smears in this study group. The 58 patients represent a combined rate for Classes III and IV of 1.7 per thousand (Table I and Figs. 1 and 2).

Evaluation of the 58 abnormal smears observed in these teen-age patients revealed the data which are detailed in Fig. 3. It is significant that 45 (76 per cent) of these patients were 18 and 19 years old.

Repeat smears were requested on all patients. Some were difficult to obtain because the postpartum patients had visited the Chicago Board of Health Clinics for the first time while in the third trimester.

Twenty of the 58 patients were referred to hospitals for histologic verification. The results of these referrals are detailed as follows: Of the 20 patients referred, seven had cytologic studies only; four smears were Class I and three were Class II. Two patients had biopsies only; five had biopsy and conization, three had conization only; three had conization and curettage. These procedures yielded two benign lesions and seven dysplasias. Of the four patients with carcinoma in situ, one had a vaginal hysterectomy with residual carcinoma in situ in the surgical specimen. She was 18 years old (gravida 6, para 3).

Of 38 nonreferred patients, 25 were lost to follow-up, nine were uncooperative, three were treated and had repeat cytology, and one had a smear which reverted to Class I.

Of the 58 abnormal cases, 47 (79 per cent) were either pregnant or postpartum. The gravidity and parity of this group are detailed as follows: Of the 58 patients, 47 had a gravidity range of one through six. Of this group of 47 patients, 35 had a parity range of one through five. The parity and gravidity for 11 of the 58 patients were classified as "unknown."

One hundred and thirty-nine Papanicolaou smears were recorded for these 58 abnormal cases by the Board of Health Cytology Laboratory and 23 smears were taken by referral hospital cytology laboratories. This is a composite total of 162 cytology smears.

Conclusion and summary

When establishing criteria for the implementation of a mass cytology screening program in a specific target population, one of the most important considerations should be age grouping. The Chicago Board of Health has reduced the number of Papanicolaou smears taken on young women under the age of 19. However, this approach does not exclude women under age 19 who epidemiologically and/or clinically warrant the implementation of a Papanicolaou smear. In Chicago, from 1962 through 1969, approximately 25 per cent of the total patient load were teen-agers under the age of 19 who were being screened annually in Chicago Board of Health Clinics. These data and our experience with the female teen-age population utilizing the Chicago Board of Health Clinics suggest that our efforts should be concentrated in the higher risk age population for Papanicolaou smear screening.

The Chicago experience reinforces the concept that epidemiologic selection of populations to be mass screened is an imperative course of action if fiscal effectiveness and responsibility are considered major objectives. It is obvious that epidemiologic factors such as degree of risk, racial and ethnic medical attitudes, socioeconomic stratification, and other demographic characteristics should be considered as important variables in determining the selective populations for all public health Papanicolaou screening programs.

It is our consensus that cervical Papanicolaou screening efforts should be focused on "high-risk" populations. This concept in no way excludes any teen-age girl from having a cervical Papanicolaou smear taken when epidemiologically and/or clinically warranted, with the use of Friedell's biologic age of cervix formula.

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Association of estrone with the development of endometrial carcinoma

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Although few clinicians suspect estrogen as the prime cause of endometrial carcinoma, a retrospective study showed the following among postmenopausal women: 70 per cent of those with endometrial carcinoma and only 23 per cent of matched control subjects used estrogen systemically. Endometrial cancer developed an average of five years earlier in those taking than in those not receiving estrogen. Forty-two per cent of those with endometrial cancer had received estrogen three years or more. Most significantly, conjugated estrogens were used by 89 per cent of postmenopausal cancer patients who had received any estrogenic substance. The increased risk of endometrial cancer for all patients receiving conjugated estrogen (the risk ratio) was 7.4. This risk ratio increased with duration of conjugated estrogen exposure, from 4.6 in patients with less than three years' exposure to 9.2 in patients with three or more years' exposure.

AMONG THE 1.1 million subscribers to the Kaiser Foundation Health Plan in Southern California, our gynecologic tumor service has noted an increase in the detected incidence of endometrial cancer while the incidence of invasive cervical cancer has diminished. At the Los Angeles Kaiser Permanente medical center, the ratio of endometrial to invasive cervical cancer is now 5.5:1. In addition, for every 3.6 patients with endometrial cancer, an additional patient with its frequent precursor, atypical endometrial hyperplasia, is treated by hysterectomy but not tallied in the tumor registry. The histologic distinction between atypical hyperplasia and carcinoma is often subtle; and since early endometrial carcinoma and severe atypical endometrial hyperplasia are treated identically, conservative pathologists tend to downgrade borderline lesions. Accordingly, the full magnitude of the shift toward endometrial carcinoma may not be appreciated from incidence data alone.

Two known factors may account for this shift. Contemporary treatment methods have mainly eliminated cervical intraepithelial neoplasia before invasion occurs, and the aging of the nation's population increases the number of women at risk of endometrial cancer. Reported here are the results of our search for a third epidemiologic factor that might contribute to the increasing incidence of endometrial carcinoma.

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Subjects and methods

During the 42 months from July, 1970, to January, 1974, the diagnosis of endometrial carcinoma was made in 94 women at the Kaiser Permanente Medical Center, Los Angeles; these 94 women comprise the patients of the study group. Two control subjects for each patient were randomly selected from a computer printout of the Health Plan subscribers in the geographic area served by this medical center, excluding women who had undergone hysterectomy. The control subjects were matched for age within one year of the patient's age, area of residence by postal zip code, and duration of Health Plan membership (the control subject had been a member at least as long as the matched patient). The control survey determined the percentage of women who had received estrogen

Table I. Average ages of 94 patients with endometrial cancer

Estrogen therapy	*Premenopausal (years) (No. = 15)*	*Postmenopausal (years) (No. = 79)*
No estrogen	43 (No. = 12)	63 (No. = 14)
Receiving estrogen	47 (No. = 3)	58 (No. = 65)
All patients	57	

Table II. Estrogen therapy of 13 premenopausal patients with endometrial cancer and 26 matched control subjects (percentages)

Estrogen therapy	*Patients (No. = 13)*	*Control subjects (No. = 26)*
No estrogen	69	96
Conjugated estrogens*	15	4
Other estrogens	8	0
Conjugated estrogens and other estrogens	8	0

*Premarin, Ayerst Labs., Div. American Home Products Corp., 685 Third Ave., New York, New York 10017.

Table III. Estrogen therapy of 81 postmenopausal patients with endometrial cancer and 162 matched controls (percentages)

Estrogen therapy	*Patients (No. = 81)*	*Control subjects (No. = 162)*
No estrogen	30	77
Conjugated estrogens*	43	10
Other estrogens	7	6
Conjugated estrogens and other estrogens	20	7

*Premarin.

Table IV. Duration of estrogen use among patients with endometrial carcinoma (percentages)

Duration (years)	*Postmenopausal (No. = 81)*	*Premenopausal (No. = 13)*	*Total (No. = 94)*
<3	17	15	17
3-24	42	15	38
Unspecified	11	0	10
Not used	30	69	35
Total	100	99	100

systemically during the study period, the duration of drug administration, and type of estrogen(s) administered.

To avoid information bias which could result from the more probing clinical history taking after the cancer had been identified, a medical records clerk requested all records from the record room, determined the date of diagnosis of disease for each patient, then concealed all information in the record from the year before diagnosis to the present. The same period, by dates, was concealed in the control subjects' records. The records were then given to an abstractor, who filled out the abstract form without knowing whether the record was that of a patient or a control subject.

Results

Endometrial carcinoma occurred before the menopause in 13 (14 per cent) and after the menopause in 81 (86 per cent) of the 94 patients.

Endometrial carcinoma developed five years earlier on the average in the postmenopausal women who received estrogen than in those postmenopausal women who did not receive estrogen (Table I).

Among the premenopausal women, 4 (31 per cent) of the patients with cancer but only one (4 per cent) of the control subjects had received estrogen (Table II). Among the postmenopausal women, in sharp contrast with the 23 per cent of the control subjects, 70 per cent of the patients with endometrial cancer had received estrogen (Table III). Fifty-one (63 per cent) of the postmenopausal patients with cancer had received conjugated estrogens (Table III).

Forty-two per cent of the postmenopausal patients with cancer had received estrogen for three years or longer (Table IV). Sixty-three per cent of the postmenopausal patients with cancer had received conjugated estrogens (Table III).

The risk ratio (the likelihood of developing cancer, given exposure to exogenous estrogen, relative to the likelihood of developing cancer without such exposure) based upon all patients receiving conjugated estrogens for periods up to 24 years was estimated to be 7.4 (Table V).

If duration of exposure correlates directly with the incidence of disease, then increased duration of exposure should be reflected directly by heightened incidence of disease. Table VI discloses that the duration of use of conjugated estrogens correlates with an increased risk of endometrial cancer. It is important to note that the risk ratio was calculated for conjugated estrogens only, not for all estrogens. Since conjugated estrogens comprised the bulk of prescribed estrogen, adequate case numbers were available only for conjugated estrogens and not for other estrogen preparations to support data showing risk of neoplasia with estrogen.

Comment

The great frequency with which estrogens are prescribed to menopausal women is explained by the goals of gynecologic management of women past the childbearing age. Gynecologists, like all primary care physicians, seek to prevent coronary atherosclerosis and osteoporosis. Because they desire the preservation of cosmetic youth and the unflagging libido of the patients, physicians have championed estrogen replacement therapy in the hope of attaining a maximal quality of life for their patients.

It is a pharmacologic truism that drugs possessing a highly beneficial therapeutic index inescapably have a low margin of safety. This maxim applies to estrogen therapy. Induction of hypercoagulation states such as pulmonary embolus and stroke, as well as diabetes and hypertension, are documented accompaniments of estrogen use; yet well-meaning physicians frequently prescribe these hormones, giving some patients estrogen even before the menopause and continuing it in some for the duration of their lives. Adverse effects may become statistically manifest only after years of exposure to exogenous estrogen. By then, many patients have become psychologically addicted to estrogen and strenuously object to its discontinuance. These chronically exposed women are uniquely jeopardized.

As this study has shown, the duration of estrogen use correlates directly with the risk of endometrial cancer. Among postmenopausal women receiving estrogen, malignant neoplasia develops five years earlier on the average than in women who do not use such hormones.

Conjugated estrogens were used by 89 per cent of the postmenopausal patients receiving some form of estrogen. Since the principal steroid component of conjugated estrogens is sodium estrone sulfate, constituting not less than 50 per cent of the steroid composition, we must look upon estrone with particular suspicion as a possible carcinogen.

Age, duration of Health Plan membership, and area of residence were matched with control and study patients. Other suspected correlates of risk of developing endometrial cancer such as lower parity, excessive weight, and older age of menopause were also evaluted. Risk ratios and confounding effects were calculated from data bearing on these variables. These other correlates of risk were essentially similar in the study and control groups. Only conjugated estrogens use stood out as a characteristic of the cancer group, differentiating it from the control group.

Other drug use was monitored. Risk ratios were calculated for diazepam, reserpine, and thyroid of 0.7, 0.5, and 0.9, respectively. Risk ratios of 1.0 or less suggest no positive association with carcinogenesis.

Table V. Risk ratio (see text for definition) for patients and control subjects using or not using conjugated estrogens*

Estrogen therapy	*Patients (No. = 94)*	*Control subjects (No. = 188)*
Receiving conjugated estrogen*	54 (A)	29 (B)
No conjugated estrogen	40 (C)	159 (D)

Crude risk ratio: $\frac{(AD)}{(BC)} = 7.4$

*Premarin.

Table VI. Risk ratio of endometrial carcinoma related to duration of conjugated estrogen* administration

Exposure to conjugated estrogen (years)	*Risk ratio*
0	1.00
1-2.9	4.6
3-24	9.2

*Premarin.

The indication for prescribing conjugated estrogens was to moderate vasomotor symptoms in 72 per cent of the cancer patients and 71 per cent of the control subjects. Estrogen therapy was not directed toward patients predisposed to endometrial neoplasia but rather for symptomatology common to both groups.

With 15 per cent of the control population receiving conjugated estrogens and the risk of developing endometrial cancer multiplied 7.4 times thereby, why have we not witnessed a profound surge of new patients with malignancy? First, our tumor registries tell us that we have, in fact, begun to note such an increase far greater than expected from Health Plan growth. Sixty-eight cases in 1971, 89 cases in 1972, and 121 cases in 1973 were diagnosed.

Second, the risk ratio is closely tied to duration of estrogen exposure. A large population must receive estrogen for years to trigger an explosion of new cases. The "estrogen forever" philosophy has fortunately not dictated the standard therapy of the menopause, with estrogen use limited to only 23 per cent of our postmenopausal population.

Third, when postmenopausal bleeding occurs, gynecologists quickly establish the presence of any

premalignant alterations by endometrial biopsy and currettage. For every 3.6 hysterectomies done for endometrial cancer at this medical center, another hysterectomy is performed for atypical endometrial hyperplasia. Hysterectomy for precursor lesions may reduce our ultimate incidence of endometrial cancer by 21.8 per cent if we assume that all atypical hyperplasias would ultimately become cancer.

Fourth, pathologists are conservative in diagnosing cancer. They realize that atypical endometrial hyperplasia and well-differentiated endometrial cancer are treated identically. Accordingly, pathologists may downgrade borderline lesions so as not to stigmatize patients with a cancer label.

Fifth, the incidence of hysterectomy in the United States is increasing.[1, 2] The standard incidence figures for endometrial cancer fail to correct for the growing number of women in the population at large who are not at risk, women who have had hysterectomy. To be meaningful, the incidence rate for endometrial cancer should be reported as the number of new cases per 100,000 female subjects with an *intact* uterus.

Cook and Dodds,[3] in 1933, Perry and Ginzton,[4] in 1937, and Gardner,[5] in 1944, demonstrated the carcinogenicity of estrogenic substances in experimental animals. The coexistence of hyperplasia and adenocarcinoma in the human endometrium first came to light as early as 1900, when Cullen[6] described and superbly illustrated what he interpreted as a noninvasive form of endometrial cancer. Taylor,[7] in 1932, confirmed that observation. Loeb,[8] Gusberg,[9] and Hertig and Sommers[10] later recognized the histologic stepping-stones from endometrial polyp through cystic, adenomatous, and atypical adenomatous hyperplasia to carcinoma in situ and endometrial carcinoma. Because either exogenous or endogenous estrogen may induce these hyperplastic changes in the noncyclic endometrium, a few investigators[11, 12] have suspected that estrogen is the trigger for malignant endometrial transformation in the genetically susceptible woman. The clinical entities infertility, diabetes, polycystic ovaries, and the "bloody" late menopause all have a common denominator: failure to ovulate. Anovulation creates the hormonal milieu of continuous low-level estrogen stimulation in which endometrial neoplasia may arise. Such an environment is also created by functioning granulosa-theca cell ovarian tumors[13–16]; endometrial cancer is associated with as many as 27 per cent of these neoplasms. Conversely, endometrial cancer is rare in the surgically castrated woman and in the girl with ovarian dysgenesis, *unless* physicians provide estrogen replacement.[17–19]

Why, then, should endometrial cancer be more rampant when the ovary ceases to secrete estrogen after the menopause? Siiteri and associates[20–22] found that the peripheral tissues of the body aromatize endogenous adrenal androstenedione to estrone. In women with postmenopausal bleeding or hyperplasia or carcinoma of the endometrium, rates of conversion of androstenedione to estrone are double or triple that of normal. Siiteri and colleagues observed that estrone production increases proportionately with body weight; thus, obesity is a major factor favoring estrone elaboration. In a lesser degree, advancing age makes an increasing contribution to heightened conversion of androstenedione to estrone. Last, Siiteri and coworkers reported that chronic liver disease blocks the conversion of androstenedione to 17-ketosteroids, resulting in higher levels of androstenedione, from which higher levels of estrone are derived. Confirming these findings, Schindler and associates[23] noted that adipose tissues taken from patients with endometrial cancer converted androstenedione to estrone at rates nearly four times those in tissues taken from control subjects without cancer. Siiteri and MacDonald[21] stated, "This strong clinical and laboratory correlation between endometrial neoplasia and the exclusive production of the weaker estrogen, estrone, is a most provocative finding meriting further study to ascertain if a causal relation exists between the two."

Smith and associates[24] and Ziel and Finkle[25] reported in December, 1975, the association of endometrial carcinoma with conjugated estrogen use.

Sodium estrone sulfate is the principal steroid component of conjugated estrogens and of esterified equine estrogens*; piperazine estrone sulfate† is the sole component of another, less commonly prescribed estrogen. High levels of estrone, either endogenously derived from adrenal androstenedione or exogenously supplied in conjugated estrogen, create an endocrinopathic environment that may trigger endometrial cancer in the genetically susceptible patient.

The endometrium is capable of converting estrone to estradiol.[26] Estradiol has ten times the estrogenic activity of estrone. This intracellular conversion of estrone to estradiol in the endometrium may account for the carcinogenicity of estrone and, therefore, of conjugated estrogens.

*Femogen, Fellows Medical Mfg. Co., Inc., Subsidiary of Chromalloy Amer. Corp., 12741 Capital Ave., Oak Park, Michigan 48237.

†Ogen, Abbott Laboratories, 14th & Sheridan Rd., North Chicago, Illinois 60064.

Conclusion

The prolonged administration of estrogen, estrone, in particular, appears to enhance the risk of endometrial neoplasia. It would seem worthwhile to withhold estrogen therapy except where its benefits clearly outweigh its known risks.

The current vogue of considering the menopause to be a disease state caused by estrogen deficiency and measured solely by the cessation of menstruation is the conceptual basis of the statement frequently made by endocrinologists: "I treat hypothyroidism with thyroid and adrenal insufficiency with cortisone. Why not treat ovarian failure with estrogen?" However, cessation of ovarian function simply denotes aging of the ovaries with depletion and atresia of the follicular apparatus. It indicates the diminution of estrogens to a level at which menstruation ceases rather than an absence of estrogen. To argue that cessation of menstruation dictates estrogen replacement is to ignore the contribution of estrone that is normally derived from adrenal androstenedione.

The endocrinologist who gives estrogen as treatment for the menopause is hoping to prevent senescence. His patient is readily deluded by her wish to preserve her figure and by her physician's implication that estrogen promises eternal youth. In a rational approach to therapy, risk is weighed against benefit. In long-term estrogen replacement therapy, particularly where estrone is used, risk may outweight benefit. The data presented here strongly suggest an association of endometrial neoplasia with estrogen administration.

The authors acknowledge the contribution of our abstractors for the study: David Harrison, Susan Lieberman, Mary Rambo, and Sandra Tyson. We recognize the assistance of Ruth Straus, who, having read a draft of this paper, injected precision into our grammar, clarity into our expression, and vitality into our presentation.

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Ventilatory and blood gas changes during laparoscopy with local anesthesia

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A study was performed on a series of healthy patients undergoing laparoscopy for tubal ligation with local anesthesia in a non–operating room setting. Ventilatory parameters, blood gas, pH, blood pressure, and pulse were monitored. The results revealed that no adverse effect on hemoglobin saturation or carbon dioxide exchange were found when fentanyl alone was used as a supplementary analgesic. An occasional vagal reflex was observed, and it is recommended that an intravenous line be established in the event that pharmacologic intervention should become necessary. Nitrous oxide produced less discomfort than carbon dioxide as the inflation gas with this minimal analgesia.

IN RECENT YEARS, laparoscopy for diagnostic procedures and tubal ligation has become a clinically useful procedure. Because of the shortage of hospital beds and ancillary personnel required for in-hospital care and general anesthesia, a study was undertaken to determine the safety of outpatient laparoscopy with local anesthesia in a non–operating room setting. The study, which was performed in the clinical research unit of the North Carolina Memorial Hospital, was designed to simulate the outpatient clinic environment and was carried out on healthy patients with local anesthesia supplemented by a short-acting narcotic analgesic. Ventilation, carbon dioxide exchange, blood gases, and pH were studied with reference to the safety of the procedure.

Methods

The subjects were 15 women, Class I (ASA), between 21 and 42 years of age who were chosen at random. All were advised of the study, and informed consent was obtained. Prior to the procedure, each patient had a medical examination, complete blood count, urinalysis, and Papanicolaou smear which were within normal limits.

The outpatients presented to the Clinical Research Unit after fasting and without premedication. An intravenous line was established. After Allen's[1] test to evaluate patency of the collateral circulation to the hand, a percutaneous arterial catheter (20 gauge) was inserted into a radial artery with local anesthesia. This was connected to a pressure transducer and used for blood pressure and pulse recording as well as arterial blood gas and pH sampling (Fig. 1).

Minute ventilation ($\dot{V}_E$), tidal volume (V_T), respiratory rate (f), and vital capacity (VC) were determined by electronic integration of air flow, measured with a pneumotachograph* and a differential pressure transducer.† The pneumotachograph was calibrated over a wide range with known volumes of room air.

The per cent of expired carbon dioxide was continuously sampled at the patient's mouthpiece and measured with an infra-red carbon dioxide analyzer.‡ End tidal P_{O_2} ($P_{ET}O_2$) was measured in samples of gas taken from the mouthpiece with the use of the synchronous pulse generator method described by Roberson and associates.[2] The patients breathed

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*Fleish No. 3, Instrumentation Associates, Inc., 17 W. 60th St., New York, New York 10023.

†Sanborn 270.

‡Godart capnograph, Instrumentation Associates, Inc.

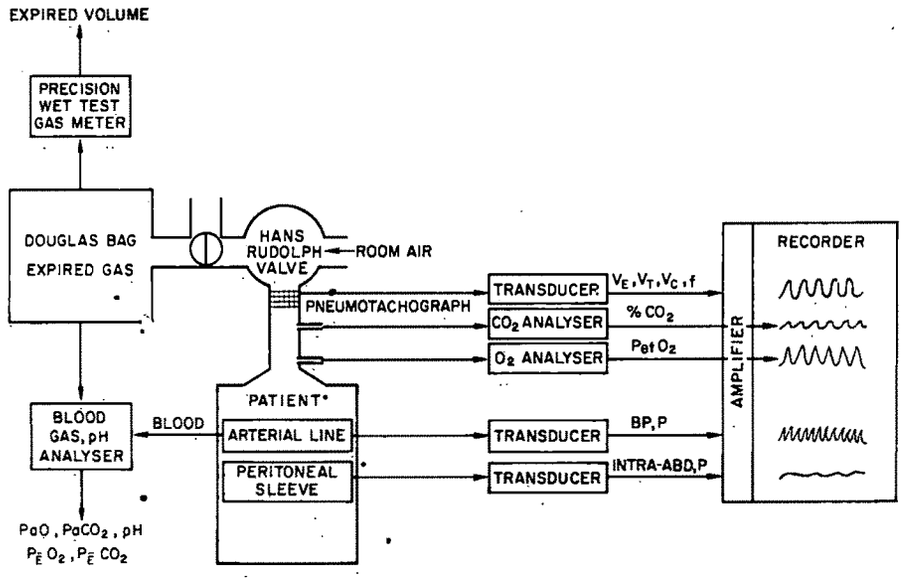


Fig. 1. Data-collecting apparatus.

through a low-resistance Hans-Rudolph valve which enabled collection of expired gas in 100 L. Douglas bags for measurement of mixed expired carbon dioxide ($P_{\bar{E}}CO_2$), oxygen ($P_{\bar{E}}O_2$), and gas volume ($\dot{V}_E$). All blood gas and expired air analyses were performed on a pH and blood gas analyzer.* Gas volumes were measured with a wet test gas meter† and corrected to standard pressure temperature, dry, and body temperature and pressure, saturated, and for the volume of expired gas extract by the carbon dioxide analyzer. Continuous blood pressure and intraperitoneal pressure, when appropriate, were measured with pressure transducers.‡ The appropriate measurements were recorded by a thermal recording system.§ Standard calculations were used for ratios of dead space to tidal volume (V_D/V_T), hemoglobin saturation (Sao_2), and carbon dioxide production.

Experimental protocol

The study consisted of five observation periods during which the experimental data were collected. Pre- and post-operative control periods were employed for collection of base-line data, and three intraoperative collection periods were chosen to coincide with times of maximal stress which were deemed most likely to compromise respiratory function and to produce measurable changes in ventilation.

The patients were allowed to rest in the supine position until they appeared calm, breathing was regular, and blood pressure and pulse rate were stable. The mouthpiece was inserted, and the patients again were allowed to rest.

Preoperative control period (supine position). During a three-minute period, expired air was collected. Duplicate blood gas samples were obtained in sealed glass syringes and stored in ice for later analysis. At the end of the three-minute period, the VC was measured in triplicate.

Period after narcotic administration (supine position). The patients were given fentanyl (approximately 2 μg per kilogram intravenously, and, when evidence of narcosis was apparent, e.g., decreased respiration, blood pressure, and pulse rate and changes in sensorium, the above measurements were repeated.

The duration of the data-collecting periods after fentanyl was administered was approximately 25 minutes—short enough so that narcosis was present during all observation periods and the patients would be reasonably comfortable.

Period after twenty-degree Trendelenberg tilt (lithotomy position). The patients were placed in the lithotomy position with a 20-degree Trendelenberg tilt, and, after five minutes, the measurements were repeated.

Period after establishment of pneumoperitoneum (lithotomy position with 20 degree Trendelenberg tilt). The pneumoperitoneum was established with carbon dioxide and nitrous oxide used as the inflation

*Model 113, Instrumentation Laboratory, Inc., 113 Hartwell Ave., Lexington, Massachusetts 02173.

†Precision Scientific Co., 3737 W. Cortland St., Chicago, Illinois 60647.

‡Model MP17, Micron Instruments, Inc., 1519 Pontius Ave., Los Angeles, California 90025.

§Series 8800, Hewlett-Packard Co., 1501 Page Mill Rd., Palo Alto, California 94304.

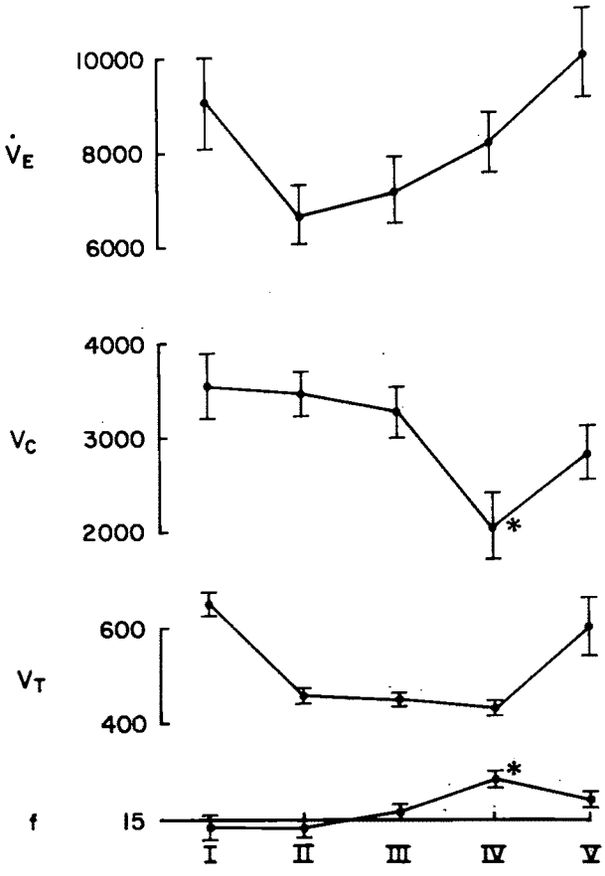


Fig. 2. Ventilatory parameters, plotted as mean values, with bars representing standard errors of the means, are $\dot{V}_E$, VC, V_T and f in each observation period. (*The t test was significant at $p < 0.01$.)

gas, and an intra-abdominal pressure of 12 to 18 cm. of water pressure was maintained. The measurements were repeated. The tubal sterilization procedure was accomplished by the laparoscopic clip method of Hulka and associates[3] with local anesthesia as described by Fishburne and colleagues.[4]

Postoperative control period (supine position). The pneumoperitoneum was terminated; the patients returned to the supine position, and the measurements were repeated.

Results

Ventilatory changes (Fig. 2). After the administration of fentanyl, the mean $\dot{V}_E$ decreased due to a decrease in V_T, and the f remained essentially the same. In the Trendelenberg position, the $\dot{V}_E$ increased slightly due to an increase in f with the V_T being essentially unchanged. After the pneumoperitoneum was established, the $\dot{V}_E$ increased because of a rise in f associated with a small decrease in the V_T. The mean VC decreased significantly. On return to the control

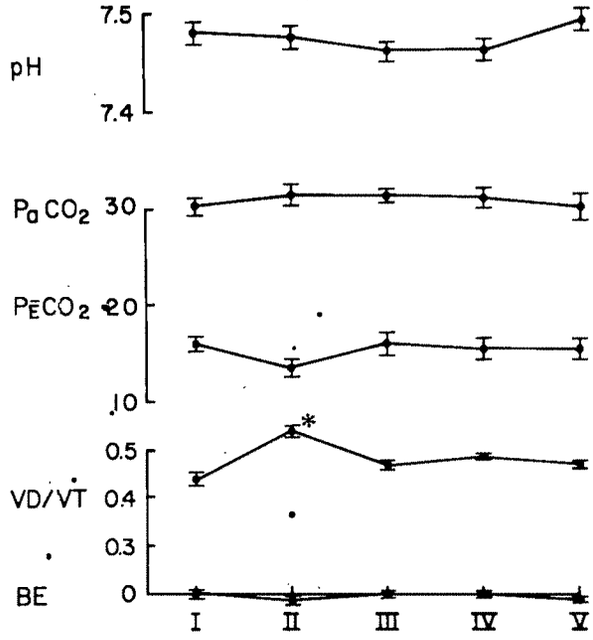


Fig. 3. Carbon dioxide exchange, plotted as mean values, with bars representing standard errors of the means, are pH, $Paco_2$, $P_{\bar{E}}co_2$ (millimeters of mercury), V_D/V_T ratio, and base excess *(BE)* for each observation period. (*$p < 0.1$, not significant.)

supine position, the mean $\dot{V}_E$ increased above the preoperative control level because of an increase in f but with a slightly smaller V_T than the original control valve. The mean VC returned to a lower value than the original control level.

Carbon dioxide exchange (Fig. 3). There were no significant changes in arterial carbon dioxide tension ($Paco_2$), pH, or base excess throughout the procedure. The mean $Paco_2$ and pH were consistent with an acute hyperventilation state. The mean mixed $P_{\bar{E}}co_2$ diminished slightly after the administration of fentanyl but returned to the control value when surgical stimulation was instituted.

The mean V_D/V_T increased after the administration of fentanyl but returned to approximately the control value during the rest of the procedure.

Oxygenation parameters (Fig. 4). The mean partial pressure of end tidal oxygen ($P_{ET}o_2$) and arterial oxygen (Pao_2) decreased after the administration of fentanyl, then gradually increased during the rest of the procedure. The mean arterial Sao_2 remained in the high normal range and never fell below 96.2 per cent saturation. The lowest Sao_2 in any of the patients was 92 per cent, and in that particular patient the hemoglobin was 13 Gm. per cent, giving an oxygen content of 18 ml. of oxygen per 100 ml. of blood, well above hypoxic levels.

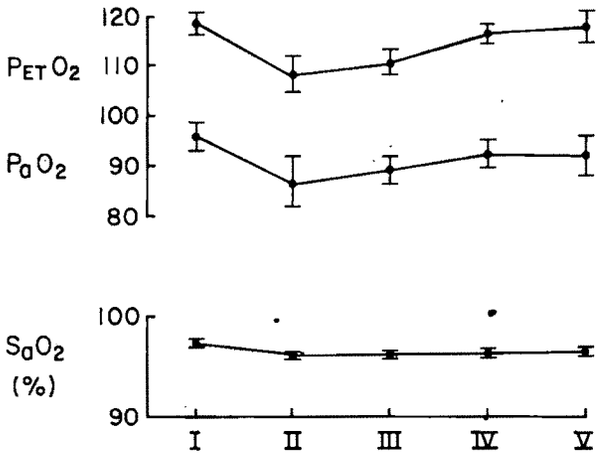


Fig. 4. Oxygenation parameters, plotted as mean values, with bars representing standard errors of the means, are $P_{ET}O_2$, Pao_2, (millimeters of mercury), and Sao_2 (per cent saturation).

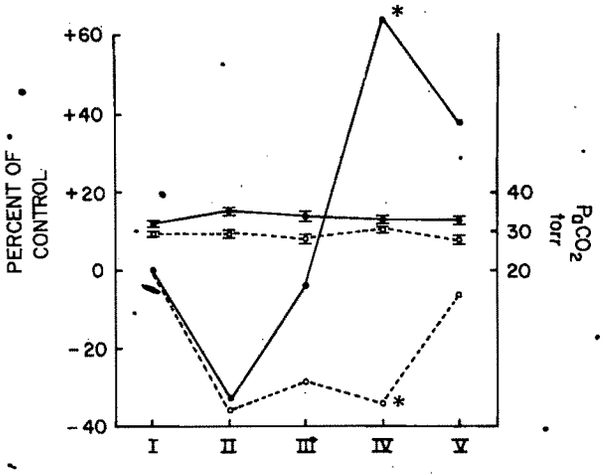


Fig. 5. Per cent change in carbon dioxide production from control values during observation periods. Continuous line is used to depict the times when CO_2 was the inflating gas. The broken line represents nitrous oxide as the inflating gas. Also plotted in millimeters of mercury (torr) are the respective $Paco_2$ values for each group. (*$p < 0.001$.)

Carbon dioxide production (Fig. 5). Carbon dioxide production (absorption) when it was used as the inflating gas was significantly greater as contrasted to the situation which obtained when nitrous oxide was used. No significant change in the $Paco_2$ was seen regardless of whether carbon dioxide or nitrous oxide was used as the inflating gas.

Comment

Although the patients appeared calm, with steady vital signs, and admitted no undue stress or anxiety when questioned, it is apparent from the $Paco_2$, base excess, and pH determinations that all were hyperventilating and exhibited acute respiratory alkalosis. The mean initial $Paco_2$ was 30 mm. Hg and never increased significantly. This is often a difficulty in using untrained volunteers for the study of ventilation while the patient is awake. The control of respiration in patients who are awake versus anesthetized patients is very different as pointed out by Fink.[5]

Another factor influencing the observed lack of change in the $Paco_2$ is the data-collecting interval. When carbon dioxide is excessively expired, body stores decrease. As ventilation decreases, it takes time to replace these stores of carbon dioxide, and the $Paco_2$ will not reflect the increase until the stores have reaccumulated and are in equilibrium with the arterial carbon dioxide tension. The time constant is four minutes, and it takes five time constants or 20 minutes to reach 99 per cent equilibrium.[6] This is made apparent in the observations after the administration of fentanyl. The mean $\dot{V}_E$ decreased 3,000 ml. per minute, but the $Paco_2$ increased only 1 mm. Hg. Since fentanyl reaches peak activity in three to five minutes, the time interval between observations was not sufficient to allow equilibrium to be obtained.

The large increase in $\dot{V}_E$ during the pneumoperitoneum was brought about by an increase in f with a small decrease in V_T even though the patients had sufficient VC to increase the V_T. However, as pointed out by Mead,[7] the principal site of the sensory end of the control mechanism for respiratory rate is in the lungs. The mechanical compression of the lungs produced by the elevated diaphragm arising from the Trendelenberg tilt and the pneumoperitoneum may have produced an increase in frequency of afferent impulses which would help to compensate for the increased work load by stimulating an increase in the rate of breathing. The optimal breathing pattern (rate and depth) is that which produces the least cost in terms of respiratory work (Otis and co-workers[8]). These patients minimized ventilatory work by increasing respiratory frequency with a resultant decrease in V_T.

Pain probably played some part in the changed pattern of breathing as suggested by the postoperative control value in which $\dot{V}_E$ was greater than the preoperative control level by virtue of an increased f but slightly smaller V_T (Fig. 2). However, our data were not acquired during the actual surgical manipulation so no data are available to substantiate any conclusions.

The V_D/V_T ratio is dependent on $Paco_2$ and P_ECO_2 and is an expression of physiologic V_D in relation to V_T. As the ratio is increased, it reflects an increase in the

fraction of ventilation which is wasted and expresses the ventilation-to-perfusion ratio ($\dot{V}/\dot{Q}$) inequality. This may be due to redistribution of inspired air to areas of low perfusion as well as redistribution of blood flow away from ventilated alveoli.

In any event, the crux of the matter is whether or not the patient can compensate for this wasted ventilation by increasing the $\dot{V}_E$ in order to maintain normal Sao_2 and $Paco_2$. The work of breathing will increase with an increased V_D/V_T ratio, but this increased respiratory work load was easily tolerated by the patients studied, and no changes in arterial oxygen saturation and $Paco_2$ were noted.

The Pao_2 and Sao_2 may be significantly reduced after the administration of narcotics.[9] In this study, the effect of the short-acting narcotic was offset by the stimulation produced by the surgical procedure and did not significantly reduce the Sao_2 below high normal levels.

Carbon dioxide is safe to use as the inflating gas because the increase in $\dot{V}_E$ during the pneumoperitoneum effectively expired the carbon dioxide absorbed from the peritoneal cavity, and the $Paco_2$ never rose significantly. However, each patient who had carbon dioxide used as the inflating gas complained of much more abdominal and diaphragmic discomfort than those in whom nitrous oxide was employed. They also had mild lingering referred discomfort for one to two hours, presumably due to the hydration of carbon dioxide to carbonic acid.

Our findings of adequate Sao_2 and normal carbon dioxide exchange differ from those of Alexander and associates.[10] We suggest that these findings differ because diazepam* was given in addition to fentanyl in their study. Diazepam is a respiratory depressant and shifts the carbon dioxide response curve.[11] It has not been determined whether the combination of diazepam and fentanyl is additive or potentiating in depressing respiration.

Three patients had significant vagal reflexes manifested by hypotension, bradycardia, and nausea precipitated by uterine manipulation. Atropine, 0.5 mg. intravenously, was used to successfully treat this situation. One patient had a mild vagal reflex which reversed itself upon cessation of the manipulation and administration of intravenous fluids. The vagal reflexes coincided with uterine manipulation and were more frequent in the retrodisplaced uterus; however, in a large series of 140 procedures,† a normal uterine position was no guarantee against vagal reflexes. The over-all incidence of vagal reflexes in the larger series was 14 per cent. It is imperative to monitor the patients closely at all times.

The patients in whom nitrous oxide was used as the inflating gas tolerated the procedure well, had no misgivings, and stated that they would recommend the procedure. The patients in whom carbon dioxide was used had much more discomfort and were not as enthusiastic about recommending the procedure.

All patients were able to be discharged from the recovery room within three hours, and four patients were sent home 45 minutes following the procedure.

*Valium, 0.5 mg., Roche Labs., Div. Hoffmann-La Roche Inc., Roche Park, Nutley, New Jersey 07110.

†Fishburne, J. I., et al.: Personal communication.

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Carbon dioxide hysteroscopy

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Hysteroscopy was performed on 75 patients undergoing a dilatation and curettage for routine gynecologic problems. For the distention of the uterine cavity carbon dioxide was used and found to be an adequate medium to establish a pneumometra. It provided good visualization and was simple and safe to use for the hysteroscopic examination. Arterial blood-gas determinations revealed no change in the P_{O_2}, P_{CO_2}, and pH with carbon dioxide insufflation. There were no complications in this series. The advantages and applications of carbon dioxide hysteroscopy are outlined.

THE DIRECT VISUALIZATION and exploration of the uterine cavity was introduced over 100 years ago and has been greatly improved with the development of fiberoptic instruments. For adequate visualization the uterine cavity, a shallow cleft, has to be distended. Various media have been used in the past. Sterile water,[10] rubber balloons,[7, 9] sodium chloride solutions,[10] 5 per cent dextrose in water solutions,[2, 8] and dextran have been tried but no one achieved general acceptance. One alternate method is to use carbon dioxide as the agent to distend the uterine cavity and establish the pneumometra.

Carbon dioxide was used for uterine and tubal insufflation by Rubin[11] as early as 1925. It was reintroduced in Europe for hysteroscopy in 1970 by Lindemann.[4–6] It has been available in the United States only recently and experience with it is limited. Carbon dioxide provides an absorbable medium for distending the uterus and achieves good visualization of the uterine cavity.

Materials and methods

The cervix is dilated to a No. 9 Hegar dilator. A vacuum adaptor (Fig. 1)* is placed against the cervix to prevent leakage of carbon dioxide. The hysteroscope (Fig. 2)* is then inserted through the adaptor and atraumatically advanced into the cervical canal. The carbon dioxide flow rate and the insufflation pressure can be predetermined with a quantity gauge in the hysteroscopy insufflator (Fig. 3),* which ensures constant flow and prevents fogging of the lens. The initial pressure setting should not exceed 80 mm. Hg, which provides adequate distention of the uterine cavity. The gas flow is interrupted once the predetermined pressure is reached, and an additional gauge continuously registers the actual pressure in the uterine cavity. Possible escape of gas through the cervix or through the Fallopian tubes will be registered in a sudden drop of pressure. The carbon dioxide flow is resumed automatically, which maintains distention of the uterine cavity during investigation. Less than 100 c.c. of carbon dioxide are sufficient for the entire procedure.

A 150-watt lamp as a power source† provided sufficient light for observation and diagnosis. A 300-watt lamp was utilized for photography. An Olympus OM-1 camera with a 100 mm. lens and an Ektachrome-film ASA160 was used for photography.

Hysteroscopy was performed on 75 patients between the ages of 22 and 58 undergoing a dilatation and curettage for routine gynecologic problems. Parity ranged from 0 to 8. The indications for the procedure are listed in Table I. The procedures were performed under general anesthesia. The endotracheal technique was used. The first 25 patients underwent blood-gas

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**Vacuum adaptor, hysteroscope, and hysteroscopy insufflator 2020S, Richard Wolf Medical Instrument Company, Rosemont, Illinois.*

*Vacuum adaptor, hysteroscope, and hysteroscopy insufflator 2020S, Richard Wolf Medical Instrument Company, Rosemont, Illinois.

†"Heavy Duty" fiber light projector D-5008-U, Richard Wolf Medical Instrument Company, Rosemont, Illinois.

Table I. Hysteroscopy

Indications for procedure	*No. of patients*
Menometrorrhagia	25
Postmenopausal bleeding	8
Laparoscopic tubal sterilization. Uncertain dates of last menstrual period.	20
Chronic pelvic pain	5
Intrauterine contraceptive device	7
Leiomyomata uteri	4
Postpartum and postabortal bleeding (repeated D & C)	1
Infertility	4
Cervical dysplasia—cone biopsy	1

determinations during the hysteroscopy or simultaneous laparoscopy.

At 5 to 7 minutes after the carbon dioxide had been started, serial arterial blood-gas determinations were performed on 25 patients. In all cases blood was drawn anaerobically from the radial artery with a heparinized syringe and was analyzed immediately for P_{O_2}, P_{CO_2}, and pH.

Fifteen patients underwent a laparoscopy simultaneously with the hysteroscopic procedure. Approximately 10 c.c. of indigo carmine was injected through the working channel on the hysteroscope into the uterine cavity. The pressure in the uterine cavity was then increased steadily and the fimbriated ends of the fallopian tubes were observed through the laparoscope for spillage of gas bubbles.

Following the hysteroscopic examination, a curettage of the uterine cavity was performed. Special attention was given to the areas of abnormal findings.

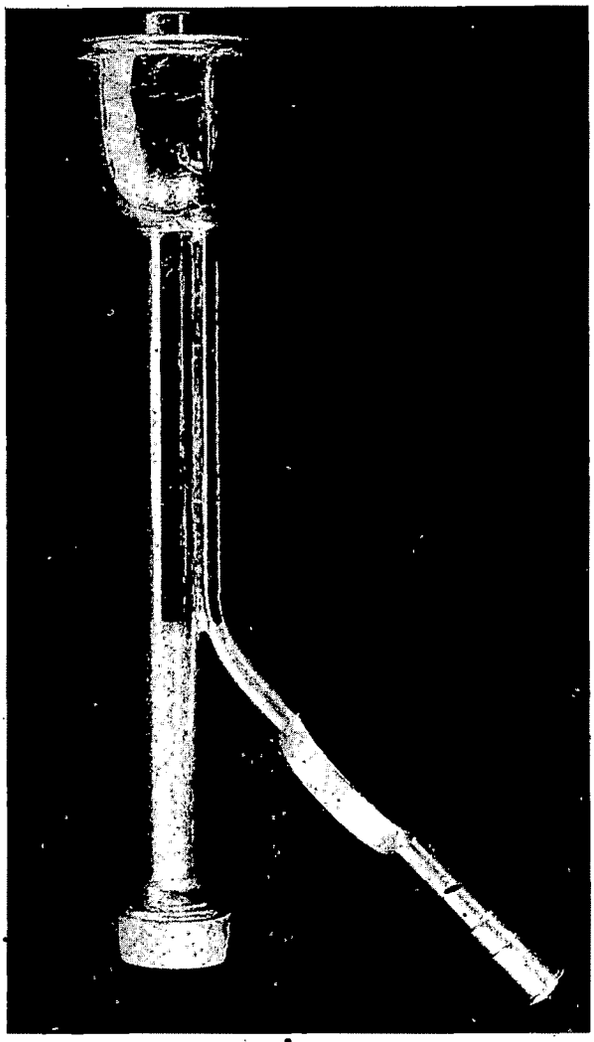


Fig. 1. Vacuum adaptor for CO_2 hysteroscopy.

Results

The hysteroscopic findings are listed in Table II. A normal uterine cavity was found in 46 of 75 patients. In seven patients undergoing a hysteroscopic examination, polyps were noted in the uterine cavity and removed.

The intrauterine contraceptive devices—mostly stainless steel bands—were located without difficulty, grasped with a forceps through the working channel of the hysteroscope, and extracted by removing the hysteroscope through the sleeve of the vacuum adaptor. Submucous leiomyomata were appreciated in three of the patients with menometrorrhagia, one patient with leiomyomata uteri, and one patient with infertility. One patient underwent a hysteroscopic procedure after a repeated dilatation and curettage for postabortal bleeding and was found to have necrotic trophoblastic tissue in the anterior fundal area. Three patients with intrauterine adhesions on hysteroscopic examination had been admitted and evaluated for pelvic pain, menometrorrhagia, and infertility. In three patients intrauterine pregnancies were diagnosed and terminated. One patient with postmenopausal bleeding was found to have an adenocarcinoma of the endometrium. A stenotic cervical os prevented the planned procedure in two patients. There were no postoperative complications in this series.

The statistical analysis of the blood-gas determinations showed no difference in P_{O_2} or P_{CO_2} in the samples taken prior to and during the hysteroscopic procedures (Table III).

Escape of carbon dioxide through the Fallopian tubes was not observed with 80-100 mm. Hg in the uterine cavity. Once the pressure was increased over 100 mm. Hg, however, the carbon dioxide did escape

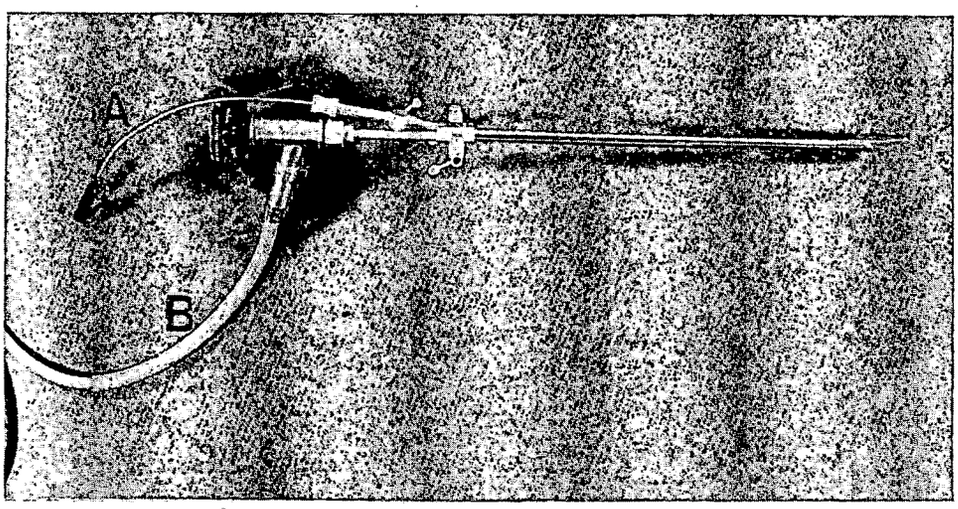


Fig. 2. Lumina-hysteroscope. Viewing angle 160°. A flexible probe *(A)* is in the working channel of the hysteroscope. A fiberoptic light-cable *(B)* is attached to the hysteroscope.

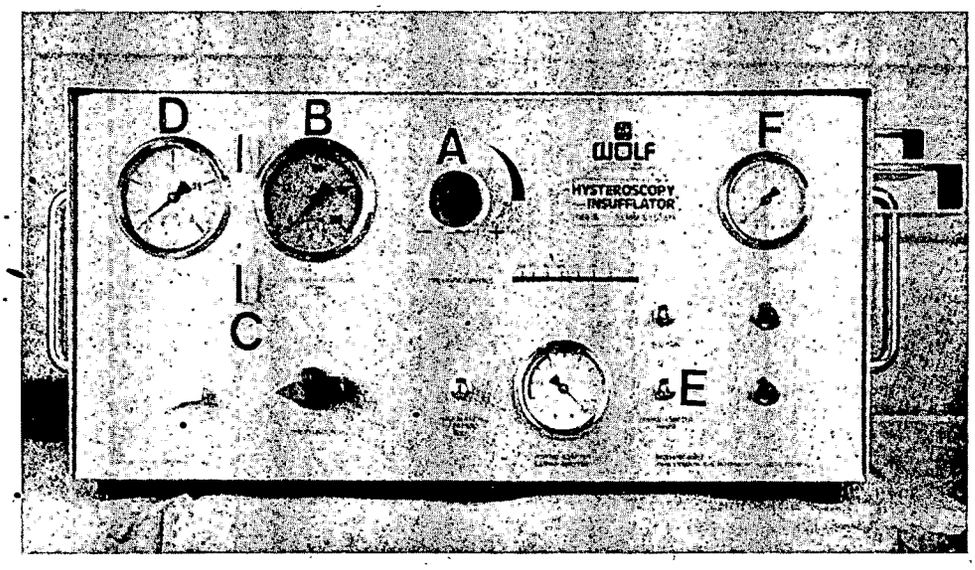


Fig. 3. Hysteroscopy insufflator 2020S. *A*, Quantity gauge with scale for pressure setting; *B*, pressure gauge for intrauterine pressure; *C*, flow-meter; *D*, gauge indicating amount of CO_2 used; *E* attachment for vacuum adaptor; and *F* supply and pressure in CO_2 tank.

in most patients with normal Fallopian tubes. This was observed through the laparoscope and also registered on the pressure gauge of the insufflator with a sudden drop in the predetermined pressure.

Shoulder pain secondary to the carbon dioxide in the peritoneal cavity did not occur postoperatively. Bleeding from the endometrium, which was occasionally encountered when the procedure was performed during the secretory phase of the menstrual cycle, decreased or subsided entirely with the increase of pressure in the uterine cavity.

The visualization of the tubal ostia was easier when the hysteroscopic procedure was performed during the proliferative phase of the cycle. A thick secretory endometrium can obstruct the tubal ostia.

Comment

For adequate visualization of the uterine cavity a variety of media and instruments have been tried in the past and are still applied. Problems arising with the use of them have prevented a general acceptance. We found that carbon dioxide provides an adequate

Table II. Hysteroscopy: Findings of hysteroscopic examinations

No. of patients	*Findings*	*Indications*	
46	Normal uterine cavity	Menometrorrhagia	14
		Postmenopausal bleeding	6
		Bilateral tubal ligation	16
		Pain	4
		Leiomyomata uteri	3
		Infertility	2
		Cervical dysplasia	1
7	Polyps	Menometrorrhagia	5
		Postmenopausal bleeding	1
		Bilateral tubal ligation	1
7	Intrauterine contracraceptive device	Intrauterine contraceptive device	7
5	Submucous leiomyomata	Menometrorrhagia	3
		Leiomyomata uteri	1
		Infertility	1
1	Necrotic trophoblastic tissue	Postabortal bleeding	1
3	Intrauterine adhesions	Pain	1
		Menometrorrhagia	1
		Infertility	1
3	Intrauterine pregnancy	Bilateral tubal ligation	3
1	Cancer of endometrium	Postmenopausal bleeding	1
2	Stenotic cervical os	Menometrorrhagia	2

Table III. Statistical analysis of blood-gas determination prior to and after 5 to 7 minutes of CO_2 hysteroscopy

Patient No.	*pH*		*PCO_2*		*PO_2*	
	Pre	*Post*	*Pre*	*Post*	*Pre*	*Post*
1	7.400	7.400	42.6	43.0	94.0	103.0
2	7.480	7.370	34.0	43.0	128.0	108.0
3	7.430	7.470	37.3	38.8	84.0	118.0
4	7.470	7.360	31.3	46.1	80.0	79.0
5	7.420	7.450	37.5	36.5	82.0	76.0
6	7.470	7.480	35.4	34.5	102.0	105.0
7	7.460	7.460	35.5	36.6	204.0	193.0
8	7.500	7.520	37.9	33.0	122.0	132.0
9	7.360	7.390	42.9	38.1	68.0	77.0
10	7.432	7.463	32.7	30.4	108.4	70.5
11	7.442	7.352	36.4	48.0	190.1	179.3
12	7.464	7.460	35.5	35.6	204.0	193.2
13	7.506	7.380	26.7	41.6	182.6	88.1
14	7.339	7.394	41.6	28.6	195.4	198.0
15	7.500	7.490	33.9	33.9	110.6	131.8
16	7.372	7.363	43.8	44.4	114.8	116.4
17	7.398	7.344	38.9	49.3	66.5	119.8
18	7.429	7.422	42.1	43.0	94.3	103.0
19	7.470	7.483	35.4	34.5	101.6	104.6
20	7.429	7.450	37.5	36.3	82.3	75.7
21	7.434	7.471	41.9	37.3	68.5	83.8
22	7.475	7.362	31.5	46.1	80.2	79.4
23	7.298	7.334	44.2	46.3	76.9	82.0
24	7.484	7.375	34.0	43.8	128.0	108.1
25	7.500	7.528	37.9	33.0	122.9	132.1
Mean	7.438	7.423	37.1	39.3	115.6	114.2
S.D.	±0.054	±0.058	±4.4	±5.8	±44.7	±39.0
	P0.193	NS	P0.151	NS	P0.793	NS

Table IV. Hysteroscopy

Indications for hysteroscopic procedures
1. Abnormal uterine bleeding
2. Unexplained infertility
3. Uterine synechia and abnormalities
4. Possible leiomyomata and polyps
5. Examination of scars in the uterus after cesarean section
6. Location of intrauterine devices
7. Biopsy of intrauterine and endocervical lesions

Table V. Hysteroscopy

Contraindications
1. Recent uterine perforation
2. Advanced intrauterine pregnancy
3. Active pelvic inflammatory disease
4. Active uterine bleeding
5. Cervical stenosis

medium to distend the uterine cavity and insures good visualization.

The risk to the patient is minimal with the use of controlled gas delivery systems. It has been alleged that fatal and near fatal accidents occurred with the use of other gas delivery systems. No complications have been reported since the introduction of the hysteroscopy insufflator with a controlled carbon dioxide output. These systems deliver not more than the predetermined amount of 60 to 80 ml. CO_2 per minute at an also predetermined pressure, usually 80 to 100 mg. Hg. With the flow rate, pressure, and amount of carbon dioxide well controlled, the risks of gas embolism are quasi nonexistent. An adequate distention of the uterine cavity is achieved and maintained. The procedure is easily performed and does not prolong routine dilatation and curettage unnecessarily. The hysteroscopic observation is usually completed within five minutes, and absorption of gas from the endometrium is minimal. Occasional gas bubbles—bubbles occur also with the use of Dextran and other fluids—do not interfere with the visibility and disappear within a short time. The amount of information obtained by direct visualization is enormous and the true nature and size of a lesion can be easily determined.

Indications for hysteroscopic examinations are listed in Table IV.

Contraindications for a hysteroscopic procedure are few and are listed in Table V.

Carbon dioxide hysteroscopy is a simple and safe method for the diagnosis of intrauterine disease. It has overcome many of the initial drawbacks of the procedure. While curettage is a blind procedure, hysteroscopy actually visualizes the uterine cavity and helps to establish the correct diagnosis.

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Dysplasia and carcinoma in situ of the uterine cervix: Prevalence in very young women (under age 22)

A one-year study in a health plan population

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An increased number of cervical epithelial abnormalities has been observed in women up to and including 21 years of age. The prevalence and incidence of dysplasia in adolescents and young women is increasing at a time when the incidence of cervical carcinoma is decreasing. We studied 247 women under age 22 with "other than negative" cytologic diagnoses identified during a one-year period (1973) in which approximately 27,500 women in this age group were screened. The prevalence rates of "other than negative" cytology and "other than negative" cytology with confirmed histopathologic abnormalities were 9/1,000 and 2.7/1,000, respectively. Repeat smears and/or tissue specimens were obtained in over 70 per cent of these cases. The majority of abnormalities identified were mild or moderate dysplasia; however eight severe dysplasias and three carcinomas in situ were identified. Fifty-nine per cent of these women had a history of one or more pregnancies. Our findings emphasize the presence of dysplasia and carcinoma in situ in young women and suggest the need for re-evaluation of the age at which initiation of the Papanicolaou smear should be considered.

CERVICAL intraepithelial neoplasia or dysplasia occurring in very young women is now recognized as a developing medical and public health problem. Ferguson[8] reported 72 "other than negative" Papanicolaou smears among a group of 1,500 women under age 19, 10 of whom had histologically proved carcinoma in situ. Other reports confirm the observation of a varying prevalence of dysplasia and carcinoma in situ in young women with differences in occurrence rate in the various communities and socioeconomic groups studies.[1–7, 9, 13–16, 19, 22, 25, 26, 29, 30]

We have studied the 1973 prevalence of "other than negative" cytologic diagnoses and the incidence of confirmed histologic abnormality and/or follow-up cytology in women under 22 years old. The population studied was drawn from a prepaid health plan population without economic or social barriers to prevent access to quality medical care. Our findings emphasize the importance of providing routine cytology studies for sexually active women, regardless of age. We consider the finding of a 9/1,000 "other than negative" cytology prevalence rate sufficient to warrant investigating this young patient population.

Material and methods

In 1973, 219,907 women of all ages were examined by the Papanicolaou smear technique (307,000 slides); 27,508 (12.5 per cent) of these women were younger than 22 years old. All were members of the Southern California Kaiser Permanente Health Plan. Cytologic

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Table I. Cytodiagnostic terminology

"Key word" category	*Some examples of descriptive cytodiagnoses*
Abnormal	Mild to moderate dysplasia of any type (keratinizing, nonkeratinizing, metaplastic, mixed)
	Severe dysplasia versus carcinoma in situ of any type
	Invasive carcinoma, cervix; adenocarcinoma; metastatic tumor; sarcoma
Inconclusive	Atypical cellular changes not explained solely by infection, repair, or prior therapy and/or cannot exclude mild dysplasia of cervix
Negative	Benign squamous metaplasia, cellular changes or metaplasia due to bacteria. Parasites (Trichomonas), fungi, herpes, and/or repair reaction
Unsatisfactory	Air dried, too few cells, too bloody, slide not properly identified at origin

Table II. Distribution of patients in population

27,508 Patients examined cytologically (12.5% of all patients)
- 247 Patients with "other than negative" cytology
  - 208 Patients with histology and/or repeat cytology
    - 118 Patients with histologic examination
      - 62 Patients with mild or moderate dysplasia
      - 8 Patients with severe dysplasia
      - 3 Patients with carcinoma in situ
      - 45 Patients whose biopsies revealed various nondysplastic diagnoses
    - 90 Patients with one or more subsequent cytology examinations
  - 39 Patients without further follow-up at this time

and histologic diagnoses by two cytopathologists at the regional centralized cytology laboratory and by 14 histopathologists were incorporated in this report. Correlations were made without any review of histopathologic specimens. Our cytology reports incorporate cytodiagnostic terminology as noted in Table I. In addition, a "key word" notation of "negative," "inconclusive," or "abnormal" was provided, since in our experience, as well as that of others, some nongynecologist physicians do not readily appreciate the diagnostic and therapeutic implications of dysplasia.[10, 11, 17, 20, 21, 24, 28]

All patients with "other than negative" cytology were recalled to the appropriate follow-up clinic or physician for further evaluation. Repeat cytology and/or random or colposcopic biopsies were obtained where appropriate and were followed by diagnostic cervical conization when clinically indicated. The interval between repeat cytology examination was at the discretion of the patient's physician. Vaginitis and cervicitis were treated if necessary. Pregnancies were managed according to established procedures for such patients. Histologic specimens were studied on slides stained with hematoxylin-eosin, with multiple levels cut routinely in patients having conization.

Results

Of the 27,508 patients under age 22 screened by cytology, 247 patients had "other than negative" cytodiagnoses (inconclusive—rule out dysplasia; mild-moderate dysplasia; severe dysplasia–carcinoma in situ; microinvasive carcinoma; invasive carcinoma). These data are summarized in Table II. Two hundred and eight patients had further cytologic and/or histologic studies. These 208 patients formed the definitive group of our study. One hundred and eighteen patients had histopathologic studies of biopsies and/or conizations, yielding 62 cases of mild or moderate dysplasia, eight cases of severe dysplasia, and three cases of carcinoma in situ. Forty-five patients had nondysplastic tissue diagnoses on biopsy (Table III). Of the patients studied by repeat cytology, 42 again had "other than negative" cytologic examinations, and 48 reverted to "negative." However, 30 of these smears with negative reversion were repeated within an eight-week period (see below). No cases of invasive carcinoma were identified in any women under age 22. Thirty-nine patients have had no known follow-up at the time of this study.

Table IV lists the age distribution of the 118 patients with tissue examination and the diagnosis. The preponderance of proved dysplasia or carcinoma in situ occurred in the group over 17 years old. Only one of the 12 patients 17 years old or younger had severe dysplasia.

The relationship between initial and subsequent cytodiagnosis, time interval between smears, and correlation with histopathology are shown in Table V. Of 56 patients with repeat smears and histopathologic studies, the initial cytology identified 36 patients with subsequent tissue-proved dysplasia, while only seven additional patients with dysplasia were identified by the second screening following an initial "inconclusive" cytology. In 21 of these 36 patients with dysplasia, the same diagnoses were made on both cytology specimens. Thirteen biopsies were negative for dysplasia after an initial "inconclusive" or "abnormal" cytology diagnosis.

Of 129 patients having two Papanicolaou smears, 60 had a diagnosis less severe in classification on the second smear. Forty-one of these 60 had repeat smears within eight weeks. Of those 77 cases with the same or a more severe diagnosis on the second smear, only 13 had been repeated within eight weeks.

Table III. Cytology-histology correlation of 118 patients under age 22

Cytology interpretation	*No. of cases*	*Histopathology diagnosis*			
		Carcinoma in situ	*Severe dysplasia*	*Mild or moderate dysplasia*	*Squamous metaplasia, trichomonas changes, herpes, cervicitis, polyp, condyloma, "no pathologic diagnosis"*
Inconclusive	34		2	14	18
Mild to moderate dysplasia	80	1	5	47	27
Severe dysplasia to carcinoma in situ	4	2	1	1	
Total	118	3	8	62	45

Table IV. Age distribution and histopathologic diagnoses

Age of patient	*No. in age group*	*Histologic diagnosis*			
		Carcinoma in situ	*Severe dysplasia*	*Mild-moderate dysplasia*	*Squamous metaplasia, Trichomonas, herpes, cervicitis, polyp, condyloma, no pathologic abnormality*
14	1				1
15	1			1	
16	6		1	4	1
17	8			6	2
18	18		2	11	5
19	26		2	14	10
20	28	2	2	10	14
21	30	1	1	16	12
Total	118	3	8	62	45

Thirteen patients had a repeat cytology smear taken simultaneously at the time of tissue biopsy. All 13 initial cytodiagnoses correlated with the proved histologic abnormality of dysplasia; however, nine of the repeat smears were interpreted as "negative." All of these 13 repeat smears had been obtained at an interval of less than eight weeks.

Of the 118 patients with tissue examinations, 73 had dysplasia or carcinoma in situ, while biopsies from 45 patients revealed a variety of nondysplastic inflammatory, reparative, or metaplastic lesions. Sixteen of 34 "inconclusive" smears were from women in whom biopsy or conization demonstrated dysplastic histology. Fifty-three of 80 patients with "mild to moderate" dysplasia and four of four patients with "severe dysplasia–carcinoma in situ" had proved dysplasia or carcinoma in situ (Table III).

One hundred and twenty-three of the 108 patients with initial "other than negative" cytology and with repeat cytology or tissue examination had a history of one or more pregnancies. The association of age and parity is shown in Fig. 1. The morphologic types of dysplasia noted in cytology in 85 nulliparous patients are shown in Table VI. Not included in our study was one case of vaginal adenosis and one case of vaginal clear cell adenocarcinoma detected by cytology. Neither cellular nor serologic studies of possible herpesvirus infection were done.

Comment

Cytology studies are indicated for all women visiting gynecologists or obstetricians. Sexually active women, whether pregnant or not, regardless of young age, must be considered to be at risk for the development of cervical carcinoma in situ and dysplasia.[1, 2, 4–9, 13–16, 18, 22, 25, 26, 29] Our detection in this one-year study of 73 patients with histologically confirmed dysplasia or carcinoma in situ and an additional 174 patients with one or more "other than negative" cytodiagnoses represents a prevalence rate of 2.7/1,000 and 9/1,000, respectively. Approximately 62 per cent of the 118 patients with tissue examinations had proved dysplasia or carcinoma in situ (Table VII). A comparison of our findings with those of previous investigators is found in Table VIII.

Many of the variations in the prevalence and

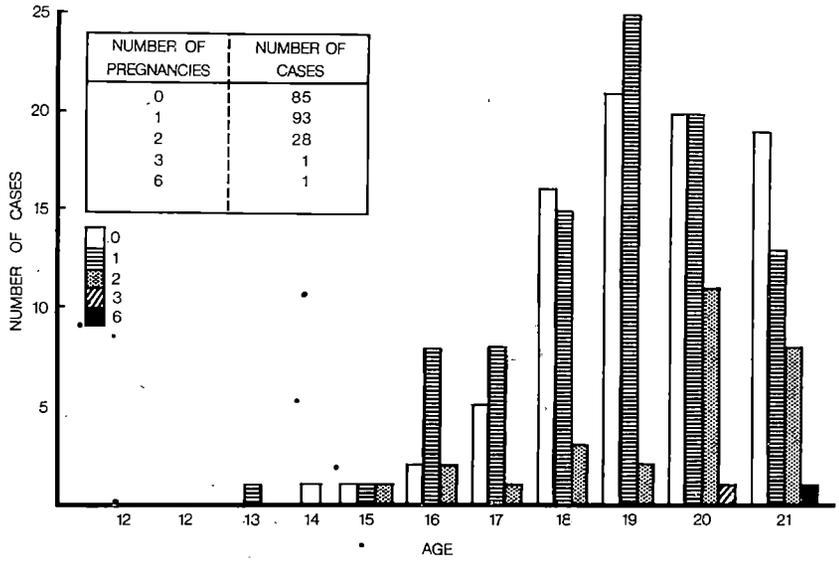


Fig. 1. Number of pregnancies per patient by age (Total: 208 cases).

Table V. Cytologic and histologic correlation related to interval between repeat cytology in 56 patients

Cytology	*Histology positive*	*Histology negative*
Repeat cytology >2 months		
First same as second	16	
First less severe than second	3	
First more severe than second	2	2
Repeat cytology <2 months		
First same as second	5	4
First less severe than second	4	
First more severe than second	13	7

Table VI. Cytologic pattern of dysplasia in 85 nulliparous patients

Dysplasia	*No.*
Keratinizing	36
Nonkeratinizing	17
Metaplastic	11
Indeterminate or mixed	21

Table VII. Summary of findings

12.5%	of all patients studied were under age 22 (27,508/219,907)
0.9%	of patients under age 22 had "other than negative" cytology (247/27,508 or 9/1,000)
0.76%	of patients under age 22 with "other than negative" cytology had repeat cytology and/or histologic study (208/27,508 or 7.6/1,000)
0.27%	of all patients under age 22 with histologically proved dysplasia or carcinoma in situ (73/27,508 or 2.7/1,000)
61.9%	of patients biopsied who were under age 22 had histologically proved dysplasia or carcinoma in situ (73/118)

incidence of "other than negative" cytology may be due to the population studied. The socioeconomic status was an important factor in several previous epidemiologic studies of cervical disease.[1, 5, 23, 27, 30] Gravidity and coital activity seem to have an important role in the pathogenesis of dysplasia and carcinoma. Findings similar to those in our 123 patients with a history of past or current pregnancy and early onset of sexual activity with multiple gravidity have been reported.[1, 2, 4–9, 13–16, 22, 23, 25–27, 29, 30] Kaufman and Leeds[14] noted that 75 per cent of their 58 patients with severe dysplasia or carcinoma in situ were parous. Wallace and Slankard[29] found that 11 of 44 patients with documented dysplasia or carcinoma in situ in their teen-age population had been pregnant one or more times. Bibbo and associates[1] found that 0.99 per cent of patients with "other than negative" cytology were 15 years old or younger and were patients at Planned Parenthood or obstetric clinics.[1]

The prevalence and incidence of carcinoma in situ and invasive carcinoma of the cervix have been decreasing in the past few decades. Linden and Dunn[18] reported a shift in detection of cervical carcinoma as carcinoma in situ from 30 per cent to greater than 90 per cent over a 10 year period. No change in the incidence of cervical carcinoma in patients 10 to 19 years old was noted in California during the years 1950 to 1969, during which time an increase in the incidence of carcinoma of the uterine corpus and vagina was noted.[18]

Several studies bear upon the predictive value of cytology, since it is apparent than an "other than negative" cytology interpretation does not imply a

Table VIII. Comparison with previous studies on dysplasia in the very young woman

	Year	*Population studied*	*Other than negative cytology*	*Tissue diagnoses*	*Severe dysplasia*	*Carcinoma in situ*	*Rate of "other than negative" cytology**	*Rate of histologically confirmed disease†*
Ferguson[8]	1961	2,300	77	56	?	10	33.4/1,000	24.3/1,000
Ferguson[9]	1967	—	167	120	?	16	—	—
Christopherson and Parker[4]	1965	5,061	—	6	1	0	—	1.1/1,000
Kaufman and associates[15]	1965	1,199	32	7	?	2	26.6/1,000	5.8/1,000
Kaufman and associates[13]	1970	10,246	302	49	11	4	29.4/1,000	4.8/1,000
Wallace and Slankard[29]	1973	7,520	51	44	?	12	6.7/1,000	5.8/1,000
Davies and Kelly[6]	1971	411	14	1	?	0	34.0/1,000	2.4/1,000
Slate and associates[25]	1972	40,000	1,325	256	?	?	33.3/1,000	6.4/1,000
Present authors	1973	27,508	247	73	8	3	8.9/1,000	2.7/1,000

*Composite rate of "other than negative" cytology: 23.0/1,000.

†Composite rate of histologically confirmed dysplasia or carcinoma: 5.2/1,000.

uniform progression for the disease process present.[10, 17, 20, 21, 24, 28] Factors affecting the accuracy of cytologic-histologic correlations include the number and capabilities of observers, the possible errors of sample and technique, interpreter bias, and the time interval between the initial "other than negative" cytology and the repeat cytology or tissue specimen.[10–12, 17, 20, 21, 24, 28] The phenomenon of "false negativity" or lesser degrees of abnormality detected on repeat cytology specimens obtained at inappropriately short intervals (less than eight weeks), while well recognized by cytologists and pathologists, is not commonly appreciated by clinicians. This may unwittingly cause a dilemma in evaluating the significance of the smear and the subsequent management of a patient with an initial "other than negative" cytodiagnosis. Our studies in these young women support those of others in noting the high rate of false negativity or lower readings on "short interval" and prebiopsy repeat smears.[10, 17, 20, 24, 28] We consider eight weeks or two menstrual cycles the minimum time interval before repeating a smear unless colposcopy, biopsy, or another procedure is deemed clinically necessary for a patient's management.

Still to be evaluated are those young women who as yet have had no further follow-up after "other than negative" cytology and those women whose repeat cytology reverted to negative, especially those repeated at short intervals. The progression-regression controversy concerning the development of significant intraepithelial neoplasia or dysplasia has yet to be resolved.[21, 24, 28] Regardless of the ultimate factors indentified as important in carcinogenesis, early exposure leads to an early risk of adverse response. Dysplasia and carcinoma in situ of the uterine cervix are prevalent in young women at a previously underestimated rate. An awareness that these lesions may occur in young women in early years of sexual activity mandates cytologic studies to identify those patients requiring careful, thoughtful, and appropriately early management.

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Ureterovaginal fistula as a complication of radical pelvic surgery

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The main objection to radical hysterectomy and pelvic node dissection as a method of primary treatment for cervical carcinoma is the inherent danger to the ureters that may result in ureterovaginal fistula formation. The purpose of this paper is twofold: (1) to show that with adoption of certain surgical and clinical measures our incidence of fistula formation has been significantly reduced, and (2) to discuss the management of ureterovaginal fistula when it occurs.

IN RECENT YEARS because of extended and widespread use of cervical cancer detection programs plus increasing public education and awareness, there has been noteworthy increase in the number of cervical carcinomas diagnosed in the intraepithelial and early invasive stages. At the Downstate Medical Center, since 1966, the number of Stage 0 cases detected annually has doubled, and the annual number of Stage I cases has increased by more than 50 per cent. Concomitant with this was a significant decrease in the number of new patients with Stages II, III, and IV cervical carcinoma.[13] This trend of increased early diagnosis of cervical cancer has also been noted by authors abroad.[15, 18] With this change in stage distribution, there was also a significant shift in age toward younger women in the age bracket from 20 to the early 40's. The treatment of patients with Stage I carcinoma of the cervix assumes greater importance as the frequency of such cases increases. Although the effectiveness of the two basic modalities of treatment for early invasive disease of the cervix is still debated laboriously, perhaps unnecessarily so, the evidence in the literature suggests equal five-year survival rates from both the primary surgical and primary radiotherapeutic approach.

The main objection to radical pelvic surgery as a method of primary treatment for cervical carcinoma is the inherent danger to the ureters.[4, 20] Radical hysterectomy requires the dissection of the entire pelvic course of the ureters which may result in the interruption of several arterial branches that provide the blood supply to the pelvic portion of the ureter. Different authors give different descriptions of the blood supply to the ureters, and Meigs,[11] in describing the steps of radical hysterectomy, pointed out the three arterial branches supplying the pelvic ureter and stressed the importance of preserving the arterial branch given off by the hypogastric artery as the blood supplies from the uterine and vesical arteries are severed during the dissection. Trauma to the ureter during radical hysterectomy and ischemia resulting in the destruction of the intrinsic blood supply located in the adventitial layer are the main causes of ureterovaginal fistula.[14] Antecedent radiation therapy and superimposed pelvic infection are predisposing factors.

Material and method

At the Kings County Hospital–Downstate Medical Center (KCH-DMC), radical hysterectomy and pelvic lymph node dissection are used to treat Stages IA2, IB, and selected Stage IIA carcinoma of the cervix (staging is based on the classification of the International Federation of Gynecology and Obstetrics, adopted

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Table I. Incidence of ureterovaginal fistula with radical hysterectomy at KCH-DMC gynecologic tumor service

Time period	*No. of radical hysterectomies for Stage I carcinoma*	*No. of ureterovaginal fistulas*	*Fistula rate (%)*	*No. of radical hysterectomies for Stage II carcinoma*	*No. of fistulas*	*Fistula rate (%)*
July 1, 1953, to June 30, 1964	117	12	10.2	49	10	20.4
July 1, 1964, to June 30, 1969	58 } 121	6	10.3 } 5.7	14 } 21	1	7.1 } 4.7
July 1, 1969, to June 30, 1973	63 }	1	1.5 }	7 }	0	0 }

Table II. Ureterovaginal fistula with radical hysterectomy at the KCH-DMC tumor service before and after modification of technique

Time period	*No. of radical hysterectomies**	*No. and % of ureterovaginal fistulas*
July 1, 1964, to June 30, 1969†	72	7 (9.7%)
July 1, 1969, to June 30, 1973‡	70	1 (1.4%)

*With pelvic node dissection.
†Before modification
‡After modification.

January, 1971).[5] Patients who are found to be poor operative risks because of life-endangering medical complications are treated by radiotherapy, as are those in the older age groups.

Table I shows the incidence of ureterovaginal fistula as a complication of radical hysterectomy and pelvic lymph node dissection in a period of 20 years at the gynecologic tumor service at KCH-DMC. The report by Calame and Nelson[4] from this institution, covering the period July 1, 1953, to June 30, 1964, showed that of 117 radical hysterectomies performed for Stage I carcinoma of the cervix there was a 10.2 per cent fistula rate, and 49 radical hysterectomies for Stage II disease resulted in a fistula rate of 20.4 per cent. From July 1, 1964, to June 30, 1973, there were 121 radical hysterectomies for Stage I disease with a 5.7 per cent fistula rate and 21 radical operations for Stage II disease with a 4.7 per cent rate of fistula formation.

Prior to June 30, 1969, after completion of the hysterectomy and node dissection, the pelvis, particularly the obturator fossa, was packed with a nonresorbable gauze head roll, wet with povidone, the outer end of which was passed out through the vaginal cuff which had been left open. This pelvic pack was left in situ from 24 to 36 hours after operation, then removed through the vagina. Reperitonealization of the pelvis was not performed. An indwelling Foley catheter was used from seven to 14 days. When continuous bladder drainage was no longer necessary, the Foley catheter was removed, and a catheter was reinserted for residual urine after two successive and spontaneous urinations. If the residual urine volume was 100 ml. or more, the Foley catheter was replaced. All patients were given antibiotics postoperatively. At this time, too, the adnexa were invariably removed.

From July, 1969, to the present, the following changes in the operative technique have been effected. After completion of the radical hysterectomy and lymph node dissection, the vaginal vault is closed with interrupted 1-0 catgut sutures. A technique similar to that of Symmonds and Pratt[19] is now used. Large plastic or rubber catheters are placed retroperitoneally into each obturator fossa and brought out through stab wounds in each lower quadrant; continuous suction is used to drain lymphatic fluid and blood that usually accumulates in these areas. Ureteral resuspension, as devised by Green,[8] is carried out so that the lower 5 to 6 cm. of the denuded ureter is sutured to the superior vesical artery by means of interrupted 5-0 chromic catgut sutures with an atraumatic needle. This replaces the ureter in a gently curving course quite similar to the normal course and anchors it adjacent to the side wall and well above the hollow of the pelvis. The position of the adjoining artery may also improve the peristaltic action of the ureter. In some instances where the ureter was unusually redundant after dissection, we have anchored it to the external iliac artery or iliopsoas muscle. Complete and meticulous reperitonealization of the pelvis is always carried out. The adnexa are removed only when diseased and in perimenopausal or postmenopausal patients. A suprapubic catheter rather than a transurethral catheter is used and has several advantages. As reported by others,[21] we have also found that suprapubic bladder drainage is better tolerated by the patients; spontaneous voiding time is shorter, and there is a lower

incidence of urinary tract infection. Furthermore, repeated catheterization is avoided as residual urine may be measured by means of a three-way stopcock before the catheter is removed completely.

Table II summarizes the over-all ureterovaginal fistula incidence in an eight-year period before and after modification of the surgical technique.

Management of ureterovaginal fistula. The clinical postoperative course of patients who developed fistulas and the method of diagnosis have been reported by Calame and Nelson[4] and further elucidated by Nelson.[14] Prominent signs and symptoms exhibited by patients who developed ureterovaginal fistula were significant fever, spontaneous leakage of urine from the vagina, and an enlarging pelvic mass. The fistula, which is usually located in the lower third of the ureter, characteristically manifests itself between the twelfth and eighteenth postoperative days. Once the leakage of urine occurs, distinction between vesicovaginal and ureterovaginal fistula can usually be made by a dye test. An intravenous pyelogram is done not so much for diagnosis but as an indicator of renal function. A ureter which is the site of a fistula may not show any abnormal changes such as hydroureter. Localization of the affected side is more difficult. Probably the best way to demonstrate the affected side is by direct observation of the ureteral orifices by cystoscopy after intravenous injection of dye. If dye-stained urine is seen spurting from one orifice and none is coming from the other, the diagnosis is clear. The most conclusive test is done at the time of surgical correction. Dye is injected directly into the ureter with a gauze sponge in the vagina. Both ureters must be tested to avoid repair of the unaffected side.

When the diagnosis of ureterovaginal fistula is established, it is generally agreed that the treatment of choice is ureteroneocystotomy,[3, 8, 14, 20] as the bladder wall furnishes an excellent blood supply to the reimplantation site. The technique of reimplantation, whether it be by end-to-side anastomosis or the valvular method, may or may not be important, particularly since we are dealing with adults.

End-to-end anastomosis of the ureter is not advocated if a ureteroneocystotomy is possible, for it may result in breakdown necrosis or ureteral stricture.[2, 9, 14] It is difficult to be precise as to the time of repair of the fistula, but most authors currently advocate a period of waiting which may range from two months (Vianna[22]) to at least six months (Orkin,[16] Talbert and colleagues,[20] and Calame and Nelson[4]).

Experience from our institution has shown that the plan of waiting for at least six months after operation has resulted in not only spontaneous healing but also better surgical repair. Prior to 1965, fistula after operation was repaired as soon as the patient's condition permitted. The operative difficulties encountered at the time were extreme with prompt breakdown of the repair in the majority of cases. After 1965, repair was done with a delay period of at least six months, provided the patients did not develop acute problems such as rapid progression of azotemia or obstructive uropathy. The operations have been easier to perform; adhesions are filmy and easily dissected to the site of the fistula, and the end results have been very good. When necessary, temporary nephrostomy is understaken to delay the operative procedure, and, during the period of waiting, the perineum is kept dry by either a Tassette cup or a Foley catheter held in the vagina by distending the balloon as large as necessary to obtain a watertight seal.

Table III. Ureterovaginal fistula as a complication of radical hysterectomy

Authors	*No. of cases*	*Fistula rate (%)*
Park and associates[17] (Washington, D. C., 1973)	150	None
Mickal and colleagues[12] (Louisiana, 1972)	64	1.6
Gitsch[7] (Vienna, 1971)	272	0.7
Averette and co-workers[1] (Florida, 1969)	65	None
Dubrotin[6] (U. S. S. R., 1963)	447	None

Table IV. Operative mortality rate with radical hysterectomy and pelvic lymph node dissection

Authors	*No. of radical hysterectomies**	*No. of operative deaths*	*Mortality rate (%)*
Liu and Meigs[10]	473	8	1.7
Brunschwig and Frick[2]	149	1	0.7
Park and associates[17]	156	1	0.6
Averette and colleagues[1]	65	0	0
Dobrotin and Bicheikina[6]	447	0	0
Symmonds and Pratt[19]	101	0	0

*With pelvic node dissection.

Comment

Recent reports as shown in Table III support the lowered incidence of ureterovaginal fistula after radical hysterectomy.

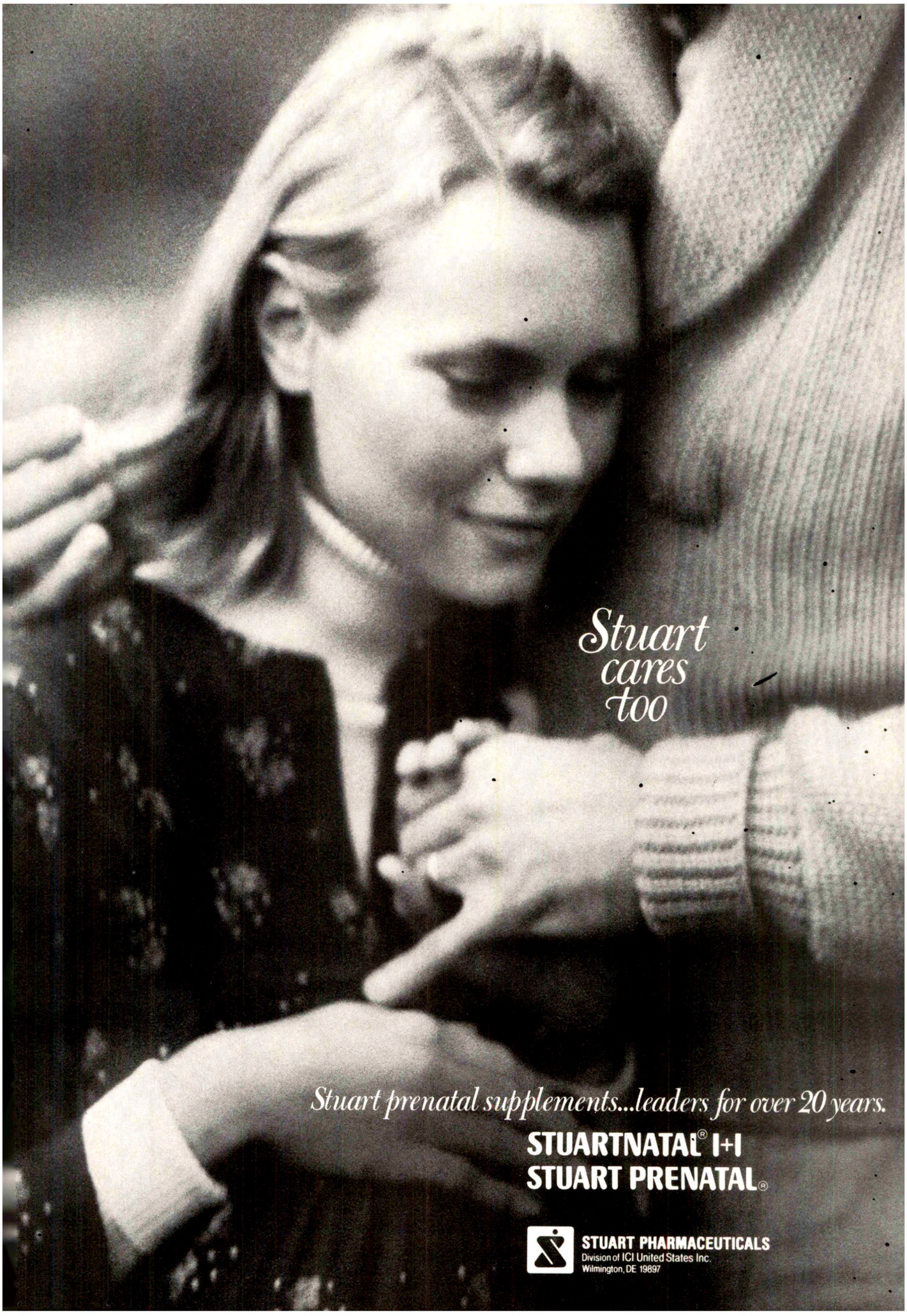
Ureteral stenosis is also a potential hazard following radical hysterectomy, and should also be borne in mind

in follow-up of patients treated by radical hysterectomy. Complications other than ureterovaginal fistula following radical hysterectomy are not significantly higher than those of other pelvic operations.[14] The operative mortality rate in most large centers in which radical pelvic surgery is common practice approaches zero. Table IV shows the operative mortality rates with radical hysterectomy reported by various authors. We have also found improvement in our mortality rate with the decreased fistula formation.[14]

The modifications in our surgical technique have not adversely affected our survival rate as the surgical steps taken in no way compromised the en bloc and radical nature of the procedure.

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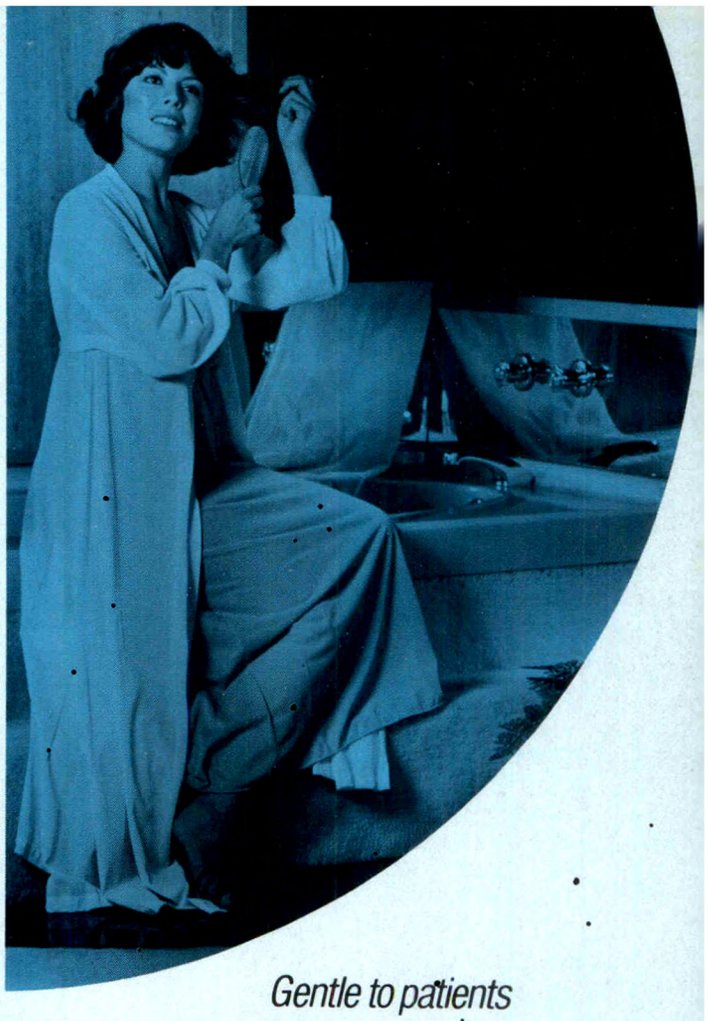
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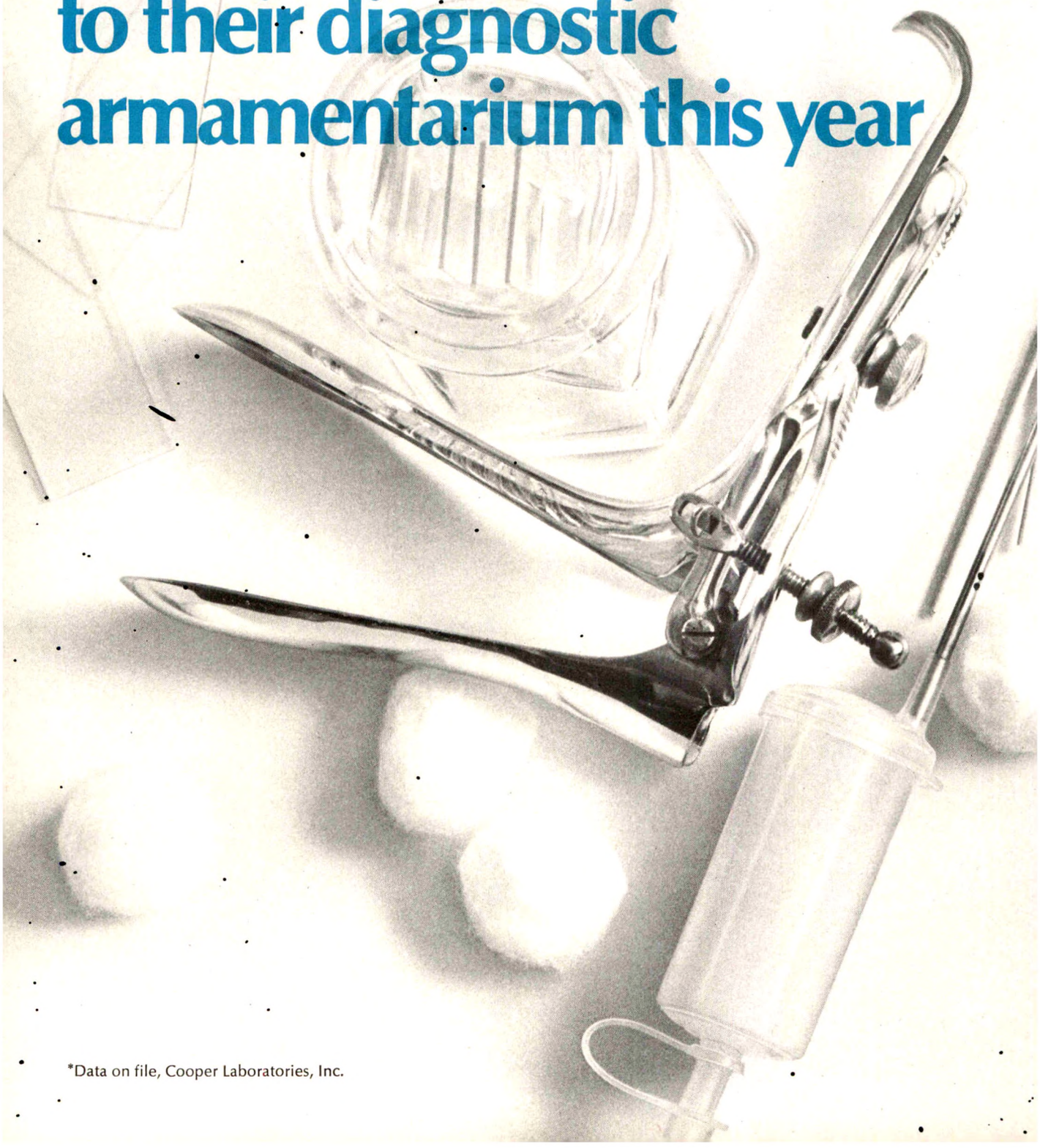
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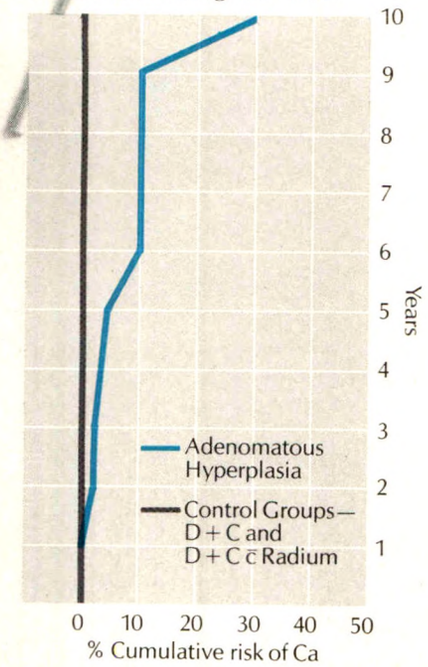


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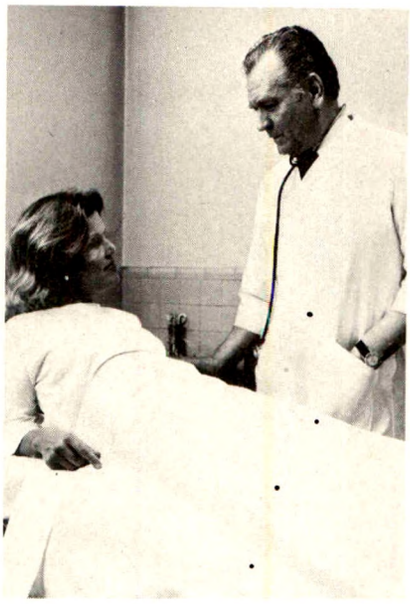
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References: 1. Gusberg, S.B.: An approach to the control of carcinoma of the endometrium, CA 23:99, 1973. **2.** Barnett, J.M.: Diagnostic suction curettage on unanesthetized patients, Obstet. Gynec. 42:672, 1973. **3.** Jensen, J.G.: Vacuum curettage, Danish Med. Bull. 17:199, 1970. **4.** Saunders, P., and Rowland, R.: Vacuum curettage of the uterus without anesthesia, J. Obs. Gynec. Brit. Commonw. 79:168 (Feb.) 1972. **5.** Walters, D., *et al.*: Office Curettage—A preliminary report of 143 cases compared with conventional curettage, Scientific Exhibit presented at Armed Forces District Meeting of A.C.O.G., Las Vegas, Nevada, Sept. 16-21, 1973. **6.** Denis, R., Jr.; Barnett, J.M.; and Forbes, S.E.: Diagnostic suction curettage, Obstet. Gynec. 42:301, 1973.

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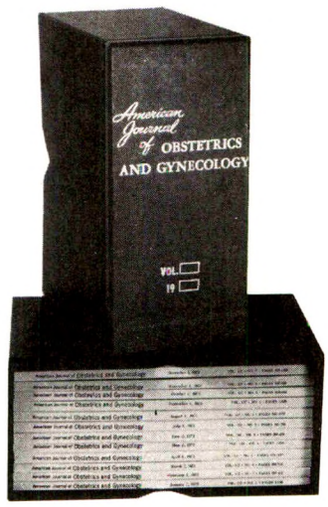
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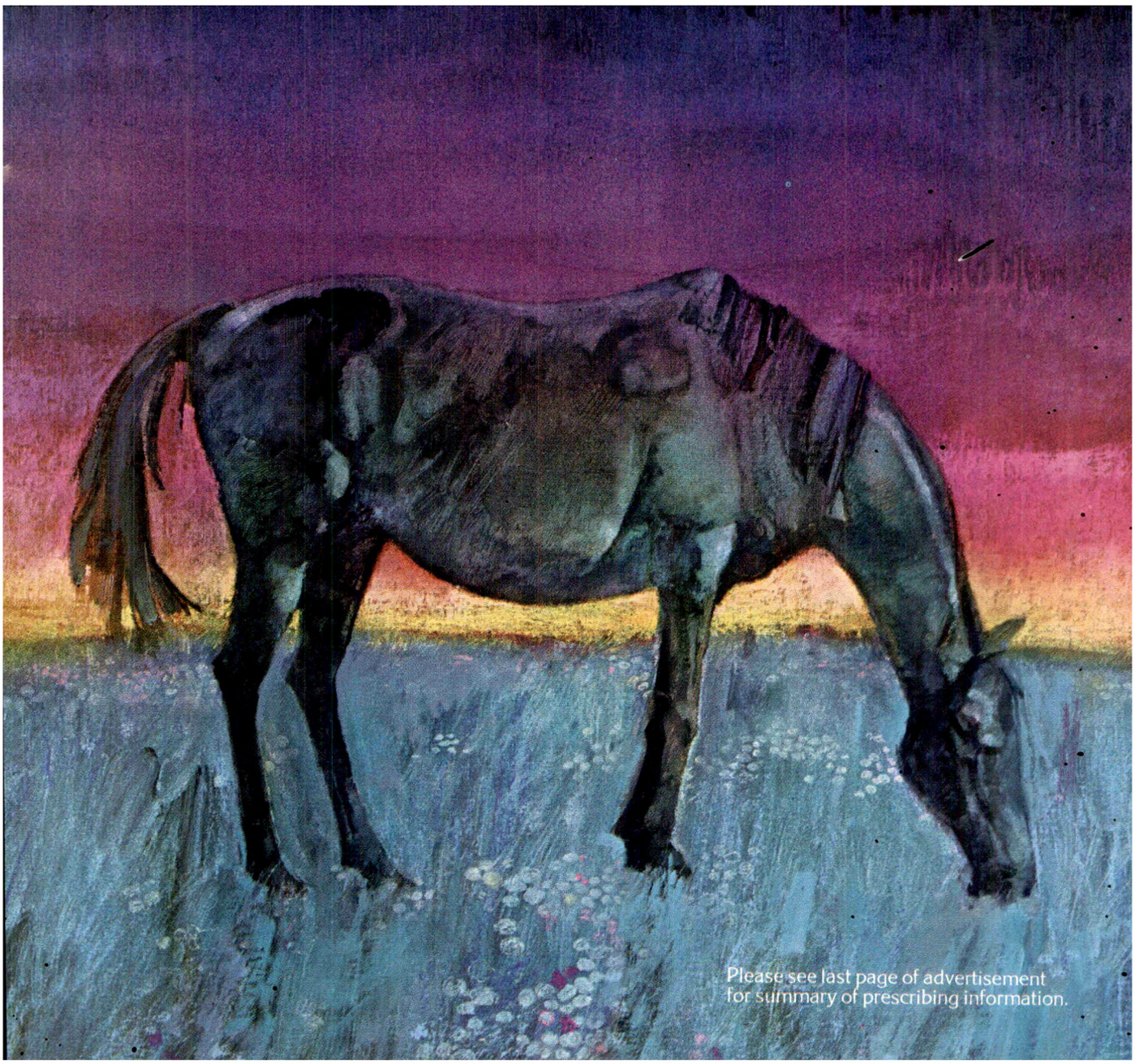
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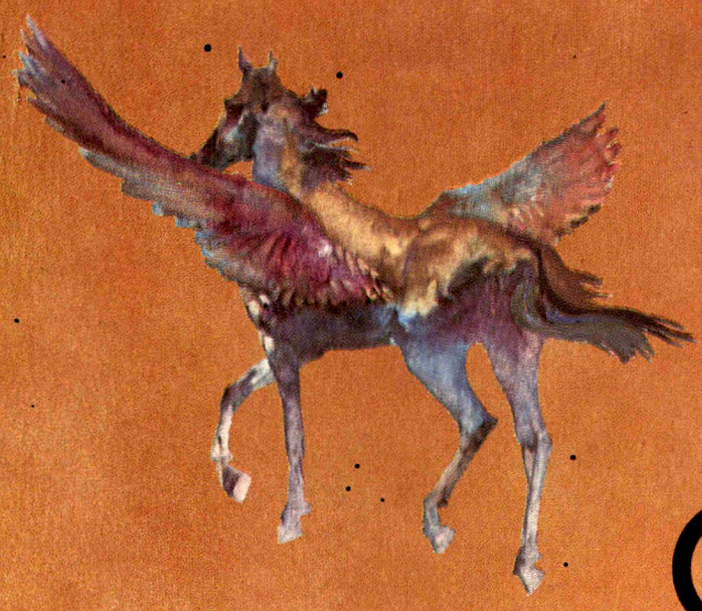
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Natural Estrogens—A Mystique

There are those who would have us believe that conjugated estrogens, U.S.P. derived from the urine of pregnant mares is endowed with a certain mystique. What, precisely, is conjugated estrogens "natural"?

A study of the excretion pattern of pregnant-mare estrogens reveals that estrone and equilin are the major components and 17 alpha-dihydroequilin a minor one. Estrone predominates in early pregnancy but as this progresses equilin levels increase and may equal or surpass those of estrone in late pregnancy. This is believed to reflect continuing changes in the placental metabolism of estrogen.[1]

The urine of pregnant mares is pooled to compensate for these variations and provide a raw material of more or less average composition. This practice, however, has the disadvantage of providing a urine pool which contains all of the excreted estrogenic and related hormones whether active or not. For example, an assay study of the estrogenic content of a "large mixture" of mares' urine reveals the following proportions: 51% sodium estrone sulfate, 22% sodium equilin sulfate, 16% sodium 17 alpha-dihydroequilin, and smaller amounts of the sodium sulfates of equilenin, 17 alpha-dihydroequilenin and 17 alpha-estradiol. Related "non-estrogenic" steroids were also present.[2]

Despite these variations, conjugated estrogens, U.S.P. is defined as containing 50 to 65% sodium estrone sulfate and 20 to 35% sodium equilin sulfate.[3]

Genisis—A Beginning

With Genisis, the beginning of a new age of conjugated estrogens, U.S.P. preparations is here. It is free of extraneous natural residues such as "non-estrogenic" steroids. It is prepared synthetically and specifically formulated so that each tablet contains the following approximate proportions of active substances:

Sodium estrone sulfate—60%
Sodium equilin sulfate—30%
17 alpha-dihydroequilin sodium sulfate—10%

Genisis is indicated for those standard indications requiring conjugated estrogens.

Genisis is supplied in snow-white pure uncoated tablets in the usual potencies of: 0.625 mg/1.25 mg/2.50 mg.


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BRIEF SUMMARY
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Indications
Genisis is indicated for replacement therapy of estrogen deficiency associated with menopausal syndrome, female hypogonadism (hypogenitalism), amenorrhea, female castration or primary ovarian failure.

Important Notes on the Use of Estrogens
In view of the positive relationship between thromboembolic disease and the use of oral contraceptives containing estrogens and progestins, it would seem prudent and in keeping with basic therapeutic principles to utilize, whenever feasible, the smallest effective dose of estrogens in treating patients.

The possible carcinogenicity of estrogens cannot be confirmed or refuted at this time. Close clinical surveillance of all women taking estrogens must be continued.

Contraindications
Patients with, or with history, of:
markedly impaired liver function;
known or suspected carcinoma of breast (except progressing disease not amenable to surgery or irradiation in women at least 5 years postmenopausal, or in men in whom castration is not possible);
known or suspected estrogen-dependent neoplasia, such as carcinoma of the endometrium;
undiagnosed genital bleeding;
thrombophlebitis, thromboembolic disorders, cerebral apoplexy.

Warnings
Discontinue medication at the earliest sign of thrombotic disorders (thrombophlebitis, cerebrovascular disorders, pulmonary embolism, retinal thrombosis), or in the presence of papilledema, retinal vascular lesions or such untoward effects as progression of cancer or hypercalcemia. Discontinue pending examination if there is sudden onset of proptosis, diplopia or migraine.

A statistically significant association has been reported between maternal ingestion of diethylstilbestrol during pregnancy and the occurrence of vaginal carcinoma years later in the offspring. Whether such an association is applicable to all estrogens is not known at this time. In any event, estrogens are not indicated for use during pregnancy.

Estrogens excreted in mother's milk may affect the nursing infant.

Precautions
Certain patients may be unusually responsive and may respond with undesirable manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding, mastodynia, edema, etc. Because of possible salt and water retention, estrogens should be used with caution in patients with epilepsy, migraine, asthma, cardiac or renal disease. Estrogens influence the metabolism of calcium and phosphorus. They should be used with caution in patients with certain metabolic bone diseases that are associated with hypercalcemia or in patients with renal insufficiency. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Diabetic patients should be followed closely because of possible decrease in glucose tolerance.

In the event of unexplained or excessive vaginal bleeding during estrogen therapy, nonfunctional causes should be borne in mind. The drug should be discontinued and a thorough investigation made as to the cause, being certain to rule out the possibility of malignancy. Pre-existing uterine fibromyomata may increase in size when estrogens are used. Therefore, patients should be examined at regular intervals throughout therapy. Women with a strong family history of cancer, recurrent chronic mastitis, or abnormal mammograms should be administered estrogens with caution. A pre-treatment physical examination should include special reference to breast and pelvic organs as well as a Papanicolaou smear since estrogens have been known to produce tumors, some of them malignant, in five species of animals.

Continuous use of estrogens will result in prolonged stimulation of the endometrium and breast. In order to avoid this, oral estrogens should be administered cyclically in the menopausal or hypogonadal patient.

The pathologist should be advised of estrogen therapy when relevant specimens are submitted. Certain endocrine and liver function tests may be affected by treatment with estrogens. If such tests are abnormal in a patient taking these drugs it is recommended that they be repeated after the drug has been withdrawn for two months. Any possible influence of prolonged estrogen therapy on pituitary, ovarian, adrenal, hepatic or uterine function awaits further study.

When large doses of estrogens are used, urinary stress incontinence may occur in nonpregnant females.

Adverse Reactions
A statistically significant association has been demonstrated between use of estrogen-progestin oral contraceptives and the following serious reactions: thrombophlebitis, cerebral thrombosis, pulmonary embolism.

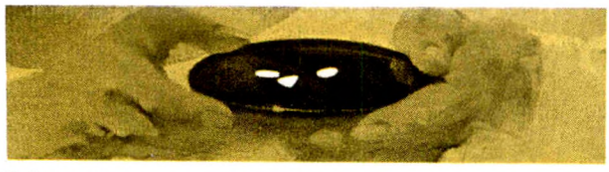
Although available evidence is suggestive of an association, such a relationship has been neither confirmed nor refuted for the following serious reactions: coronary thrombosis, neuro-ocular lesions (e.g. retinal thrombosis and optic neuritis).

The following adverse reactions are known to occur in patients receiving estrogens: nausea, vomiting, anorexia, gastrointestinal symptoms (such as abdominal cramps or bloating), edema, breakthrough bleeding, spotting or withdrawal bleeding, breast tenderness and enlargement, change in body weight (increase or decrease), headache, increased cervical mucus, allergic rash, loss of libido and gynecomastia in the male, reactivation of endometriosis, aggravation of migraine headaches, hepatic cutaneous porphyria becoming manifest, cholestatic jaundice, rise in blood pressure in susceptible individuals, mental depression, cystitis-like syndrome, loss of scalp hair, erythema nodosum, hemorrhagic eruption, premenstrual-like syndrome, changes in libido, changes in appetite, nervousness, dizziness, fatigue, backache, erythema multiforme, itching, possible diminution in lactation when given immediately post-partum, irritability, malaise.

Dosage and Administration
Dosage requirements will vary with the severity of menopausal symptoms. Initially, doses ranging from 1.25 mg to 3.75 mg daily may be required to bring symptoms under control. When this has been accomplished dosage should then be gradually decreased until the minimal effective maintenance dose is established. This will usually be 0.625 mg or 1.25 mg daily or possibly less.

Caution
Federal law prohibits dispensing without a prescription.

How Supplied—0.625 mg tablets—bottles of 100 and 500
1.25 mg tablets—bottles of 100 and 500
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References
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2. Howard, R.P. and Keaty, E.C.: Evaluation of equilin 3-monosulfate and other estrogens. Arch. Int. Med. 128:229, 1971.
3. Goodman, L.S. and Gilman, A.: The pharmacological basis of therapeutics. New York, MacMillan Pub. Co. Inc., 1975, p. 1431.

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CURRENT INVESTIGATION

This section offers prompt first announcement of new observations or discoveries. Articles should be limited to 1,500 words and 6 references. Illustrations or additional references require a proportionate reduction in total words.

Nuclear chromatin determination in amniotic fluid cells for prenatal sex prediction in the macaque

ARTHUR L. HERBST, M.D.
PRISCILLA D. TAFT, M.D.
STANLEY J. ROBBOY, M.D.*
Boston, Massachusetts

Amniotic fluid cells obtained after the ninetieth day of gestation in the macaque were analyzed for nuclear chromatin. The technique proved reliable for the prediction of fetal sex.

IN THE COURSE OF investigation of the effects of intrauterine exposure to diethylstilbestrol (DES) on the female macaque fetus, it became useful for us to establish the sex of the offspring prior to birth in order to concentrate experimental efforts on the female fetus. For many years, it has been known that a female sex chromatin body (Barr body) can be identified in the somatic cell nuclei of many mammalian species.[1] The technique for its detection in amniotic fluid cells for the purpose of prenatal sex prediction in the human subject has also been described.[2] However, we can find no study describing the usage of this technique in the subhuman primate.

From Vincent Memorial Hospital (Gynecology Service of the Massachusetts General Hospital), the James Homer Wright Pathology Laboratories, Massachusetts General Hospital, and the New England Regional Primate Research Center, Harvard Medical School.

Supported in part by Grant 13139-02 from the National Cancer Institute and Grant ET-52 from the American Cancer Society.

Reprint requests: Dr. Arthur L. Herbst, Department of Gynecology, Massachusetts General Hospital, Boston, Massachusetts 02114.

**Junior Faculty Fellow of the American Cancer Society.*

Material and methods

Pregnant animals (*Macaca mulatta* and *Macaca fascicularis*) housed in the breeding colony at the New England Regional Primate Research Center were injected with DES (5 mg. in 0.5 c.c. of cottonseed oil, intramuscularly, daily) or 0.5 c.c. of the diluent alone, beginning at Day 35 to 40 of gestation and continuing for 100 days.

Amniocentesis was performed on a total of 15 animals from both the experimental and control groups after the abdomen was surgically prepared, shaved, and cleaned with soap, iodine, and alcohol solutions. (This extensive skin preparation was adopted after one animal developed intrauterine sepsis early in the study.) The superior margin of the

Table I. Comparison of nuclear chromatin determination

Nuclear chromatin (%)	*Day of amniocentesis*	*Prenatal medicine*	*Results, gestation day*	*Offspring status*
1	88	DES	Aborted, day 115	None
2	76	DES	Aborted, day 86	None
2	100	DES	Male, 161	Living and well
3	92	DES	Male, 156	Dead, intrauterine infection
4*	135	Control	Male, 171	Living and well
5	94	DES	Male, 143	Living and well
6	110†	Control	Male, 144	Living and well
24	148	Control	Female, 160	Living and well
25	112	DES	Female, 171	Dead one day after cesarean section for prolonged gestation
25	162	DES	Female, 164	Living and well
28	105	Control	Female, 157	Living and well
30	92	DES	Female, 165	Living and well
32	109‡	DES	Female, 165	Living and well
33	163	Control	Female, 170	Living and well
35	110§	DES	Female, 156	Living and well

*M. *fascicularis*.
†Cells not interpretable on Day 82.
‡No fluid obtained on Day 54.
§Cells not interpretable on Day 81.

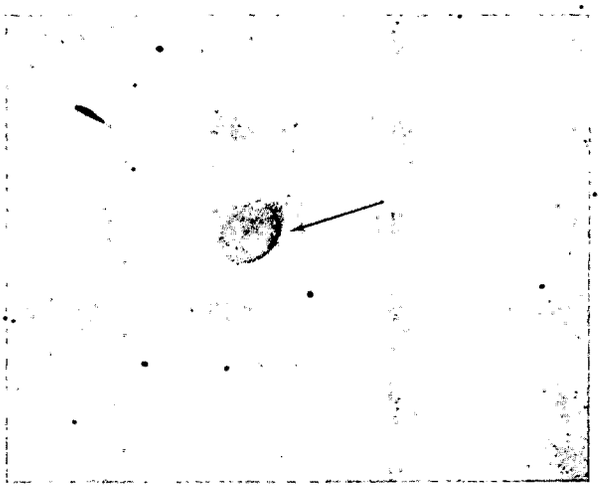


Fig. 1. Sex chromatin body (arrow) in amniotic fluid cell. (Original magnification ×2400.)

pregnant uterus was palpated and held firmly while a sterile No. 22 gauge needle was introduced halfway between the top of the uterus and the symphysis pubis. Approximately 0.5 to 1 c.c. of amniotic fluid was then withdrawn.

The amniotic fluid cells were collected on a membrane filter, fixed in 95 per cent ethyl alcohol, and stained by the Feulgen method. At least 100 cells were counted and evaluated for nuclear chromatin (Fig. 1).

Results

The Barr body count of eight offspring that proved to be female varied between 24 and 35 per cent (Table I), while the count of five male offspring varied between 2 and 6 per cent. Amniocenteses performed on two additional animals yielded sex chromatin counts of 1 and 2 per cent, but the pregnancies were subsequently aborted, and no fetuses could be identified.

During the course of amniocentesis, the bladder was occasionally entered if the needle was introduced too close to the symphysis pubis. Furthermore, it was found that amniotic fluid could not be obtained if the procedure was attempted before the tenth or eleventh week of gestation. The two specimens that were obtained before the ninetieth day of gestation (Days 81 and 82) contained cells that had degenerated and were not satisfactory for the analysis of nuclear chromatin.

Comment

The data indicate that the determination of nuclear sex chromatin in amniotic fluid is a reliable technique for prenatal sex prediction in the subhuman primate. We had initially thought that specimens with 20 per cent or more nuclear chromatin bodies were consistent with a female gestation, and those with 4 per cent or fewer, with a male gestation, as in the human subject.[3, 4] However, in this study two of the five male offspring had counts above 4 per cent (5 and 6 per cent, Table I).

These values were well below the value for female offspring of 20 per cent, and the slight increase over the expected number of positive cells might have been due to contamination with maternal cells.

Except for the introduction of intrauterine infection in one case, amniocentesis had no perceptible effect on the outcome of pregnancy in this small group of cases. In the two years prior to the present study, the *M. mulatta* breeding at the New England Regional Primate Research Center achieved 124 pregnancies with 15 losses (abortions or stillbirths). If the one case of preventable infection in the current study is excluded, there were two pregnancy losses among the 15 *M. mulattas* who underwent amniocentesis, results roughly comparable to the experience of the breeding colony in general. We have no explanation for the unsatisfactory cell preparations that were obtained when amniocentesis was done on Days 81 and 82, since at this time the cells theoretically should be satisfactory for the determination of nuclear chromatin. In view of these two unsatisfactory results, we have not subsequently initiated amniocentesis until after the ninetieth day of gestation.

The authors wish to thank Ms. Regina Dagata for her technical assistance in carrying out this project.

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Slowing of active labor associated with internal fetal monitoring

ROBERT J. SOKOL, M.D.
IVAN ZADOR, M.S.
MORTIMER G. ROSEN, M.D.
Cleveland, Ohio

Active labor progress is generally believed to be linear. By using an ultrasonic cervimeter, which continuously records dilatation of the uterine cervix, acute decreases in active-phase dilatation followed by less rapid recoveries are found to be associated with intravaginal obstetric manipulations. These findings support the concepts that active labor may be nonlinear and regression in cervical dilatation is a real phenomenon.

PROGRESS IN ACTIVE labor during the phase of maximum slope is generally thought of as being linear. This appears to be confirmed by examination of Friedman curves derived by averaging large numbers of labors. However, in 45 high-risk labors, evaluated in our initial studies of computer diagnosis of labor progression, 16 brief nonlinearities were identified during active labor. These were either short episodes of protracted active-phase dilatation, a low slope in active labor, or "shoulders," during which there was no change or a decrease (regression) in dilatation for less than two hours. Fifteen of these 16 were found to be temporally associated with simultaneous descent abnormality, amniotomy, narcotic analgesia, epidural anesthesia, or placement of internal monitors.[1] Because of these findings, we have used point-to-point labor diagnosis and evaluated the slope between each pair of successive vaginal examinations as a "finer" method of labor assessment than evaluation of smoothed curves.

Since digital examination is intermittent, with reproducibility in the range of ±5 to 10 per cent and cervical dilatation might not change very much over short periods, the reliability of our original observations could be questioned. However, with an instrument which continuously determines cervical dilatation with an accuracy of ±2 mm. from the transit time of ultrasound signals between piezoelectric crystals clipped at the three and nine o'clock positions on the uterine cervix,[2] it is possible to measure more accurately small changes in cervical dilatation, which may be associated with specific intrapartum events.

It is our purpose in this communication to present an example of continuously recorded cervical dilatation from a labor during which the fetus was electronically and biochemically monitored and to comment on the associated nonlinearity of active labor.

From the Perinatal Clinical Research Center and the Department of Obstetrics and Gynecology, Cleveland Metropolitan General Hospital/Case Western Reserve University.

Supported in part by United States Public Health Service Grants 5M01-RR00210 and HD05566-01A1 and by the Cleveland Metropolitan General Hospital Foundation.

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Case report

A 20-year-old nullipara, with a benign antepartum course, entered the hospital at term in early latent labor with fingertip cervical dilatation and the vertex at −2 to −3 station. The labor pattern is shown graphically in Fig. 1. At 1226 hours *(A)*, amniotomy was performed, and oxytocin stimulation and internal fetal monitoring were begun. At 1445 hours *(B)*, after fetal bradycardia, a scalp microblood examination was performed (pH = 7.40; base excess = −4.9). At 1540 hours *(D)*, it was necessary to reapply the scalp electrode. Labor then progressed smoothly, with spontaneous rotation of the occiput from posterior to anterior and without evidence of fetal distress. At 1856 hours, the patient was delivered spontaneously of a 3,560 gram female infant with Apgar scores of 9 and 10 at one and five minutes, respectively.

Examination of the labor graph, based purely on clinical examinations (Fig. 1), shows that protracted active-phase dilatation (0.57 cm. per hour) occurred

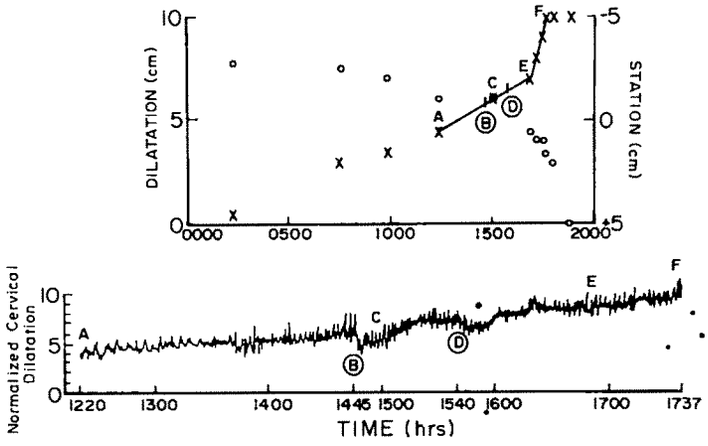


Fig. 1. Graph of a nulliparous labor (top) in occiput posterior position. × = Cervical dilatation; O = station. Interval covered by segment of continuously recorded cervical dilatation tracing (bottom) is shown by solid line. The scale of the continuous record has been normalized so that clinical full dilatation equals exactly 10 cm. At *A*, cervical dilatation was 4 to 5 cm.; amniotomy was performed, and monitoring was begun. At *B*, a fetal scalp microblood examination was performed. At *C*, cervical dilatation was 6 cm. At *D*, the scalp electrode was reapplied. At *E*, cervical dilatation was 7 cm. Interval *A* to *C* to *E* represents protracted active-phase dilatation. Interval *E* to *F* represents normal progress to full dilatation (see text).

from 1226 to 1650 hours (*A* to *E*, 4.5 to 7 cm.). Point-to-point analysis shows that the slope from *A* to *C* was 0.58 cm. per hour and that from *C* to *E* was 0.55 cm. per hour. The continuous cervical dilatation record documents what actually happened. During the fetal scalp microblood examination *(B)*, dilatation decreased acutely by 1 cm. (slope = −20 cm. per hour) and required 45 minutes to recover fully (slope = 2.4 cm. per hour). Similarly, a little later, when the scalp electrode was reapplied *(D)*, dilatation again decreased acutely and recovered more slowly.

Comment

Point-to-point analysis of labor graphs based on digital vaginal examination suggests that active labor is not immune to slowing associated with obstetric interventions. These interventions might include, for example, amniotomy, analgesia, conduction anesthesia, and intravaginal fetal monitoring manipulations. The continuous cervical dilatation record supports the concepts that progress in active labor may be nonlinear and that regression in cervical dilatation is a real phenomenon; it cannot always be dismissed as resulting from inconsistent vaginal examination. In this case, the long-term picture of protracted active-phase dilatation was produced when cervical dilatation decreased acutely and recovered more slowly with a fetal scalp microblood determination and again with reapplication of the scalp electrode.

Also, in this labor, the dilatation abnormality was associated with an apparent delay in descent. This and several other similarly monitored cases suggest that such short-term decreases in cervical dilatation may be related to loss of good application of the fetal presenting part to the cervix, as when the vertex is pushed up in the process of fetal scalp microblood sampling.

Finally, our past experience suggests that delays in the active phase are more likely to occur in labors complicated by malposition, as in this case, or by disproportion. Here, analgesia and conduction anesthesia were avoided, and normal dilatation and spontaneous delivery occurred. Since it appears that factors adversely affecting labor progress tend to be cumulative, inappropriate interventions might have resulted in true secondary arrest of dilatation. We would speculate that when internal fetal monitoring is clinically indicated, other interventions, such as simultaneous analgesia or conduction anesthesia, should be avoided. Further investigation of active-phase slowing is warranted.

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Ovarian hemorrhage complicating warfarin sodium anticoagulant therapy

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Three cases of women in the reproductive age group who received warfarin sodium therapy for pulmonary embolism are presented. The therapy was complicated by rupture of ovarian cysts with intraperitoneal hemorrhage necessitating exploratory laparotomy. The possibility of intraperitoneal hemorrhage must be considered in patients who present with abdominal pain and a history of anticoagulant therapy. Lack of awareness of the complication may result in delay in making a correct diagnosis and instituting appropriate therapy.

THE VALUE OF anticoagulant therapy for the treatment and prevention of thromboembolic phenomena is widely accepted. Hemorrhagic complications of anticoagulant therapy occur despite precautions.[1] Although the most common site of bleeding secondary to anticoagulants is the urinary tract,[2] bleeding into the ovary with the formation of a hemorrhagic cyst can occur. These cysts may subsequently rupture with intraperitoneal hemorrhage.

Three cases of patients with pulmonary embolization were given long-term warfarin sodium anticoagulant therapy. They developed ruptured hemorrhagic cysts of the ovary with intraperitoneal hemorrhage requiring exploratory laparotomy.

Case reports

Case 1. A 28-year-old, black woman, para 1-0-0-1, was admitted to the gynecologic ward because of lower abdominal pain. Two years previously, she had an inferior vena cava ligation for repeated pulmonary thromboembolism and was subsequently given warfarin sodium because of chest pains suspected to be due to recurrent pulmonary thromboembolism. On admission, a left cystic 5 by 6 cm. ovarian mass was palpated. The patient had an exploratory laparotomy. The left ovary was occupied by a 5 by 7 by 7 cm. cystic mass. A left oophorectomy was performed. She was well when discharged and was followed in the gynecology and medical clinics where she was given warfarin sodium because of recurrent thrombophlebitis. The patient was readmitted to the gynecologic ward because of lower abdominal pain. Pelvic examination revealed a right adnexal mass and a full pouch of Douglas. Culdocentesis yielded 15 ml. of unclotted blood. A clinical diagnosis of hemoperitoneum was made. An exploratory laparotomy revealed that the right ovary was replaced by a 4 by 5 by 6 cm. hemorrhagic cyst, and 300 ml. of free blood was present in the peritoneal cavity. Because the patient previously had a left oophorectomy, a subtotal hysterectomy and right salpingo-oophorectomy were performed. The patient's condition had improved when she was discharged, with follow-up to be performed at the gynecology clinic. The pathologic diagnosis of the submitted surgical specimen was hemorrhagic cyst of the ovary.

Case 2. A 38-year-old, black patient, para 1-0-1-1, was admitted to the gynecologic ward on July 22, 1974, with lower abdominal pain. She had pulmonary embolization in June, 1973, and was given warfarin sodium and followed by the medical service. She had a left salpingectomy for an ectopic pregnancy ten years previously. On pelvic examination, a 6 by 7 cm. right cystic ovarian mass was found. Exploratory laparotomy was performed on July 23, 1974, and there was 100 ml. of free blood in the peritoneal cavity. The right ovary contained a 4 by 5 by 7 cm. cyst, filled with blood. A right ovarian cystectomy was performed. The pathologic diagnosis of the specimen submitted was hemorrhagic corpus luteum cyst of the ovary. On the sixth postoperative day, the patient complained of left parasternal chest pain. A lung scan and blood gas analyses suggested recurrent pulmonary embolism. Inferior vena cava ligation was suggested; however, the

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patient refused the operation. She was then given heparin and subsequently warfarin sodium. The patient was discharged on August 12, 1974, after her condition improved. Warfarin sodium was continued, and she was to be followed in the gynecology and medical clinics. She was readmitted to the gynecologic ward on September 14, 1974, because of the sudden onset of lower abdominal pain. Pelvic examination showed lower abdominal distention and a pelvic mass, 7 by 8 cm., in the right adnexal area. The uterus was difficult to delineate but appeared to be of normal size and separate from the mass. After the prothrombin time returned to normal with vitamin K administration and blood transfusion, and exploratory laparotomy was performed. In the peritoneal cavity was 800 ml. of free blood. The left ovary contained a 4 by 3 by 6 cm. ruptured hemorrhagic cyst, and left ovarian cystectomy was performed. The patient had an uneventful postoperative course and was discharged and followed in the gynecology clinic. The pathology diagnosis of the submitted surgical specimen was hemorrhagic cyst of the ovary.

Case 3. A 27-year-old, black patient, para 1-0-0-1, admitted with lower abdominal pain, dizziness, and shoulder pain. Six months previously, the patient had a cesarean section for cephalopelvic disproportion. On the fifth postoperative day, she experienced chest pain. A lung scan revealed areas of decreased perfusion in the apex of the right lung. Anticoagulation therapy with heparin and warfarin sodium was started. The patient's condition improved, and she was discharged on the twelfth postoperative day, to be followed in the medical and postpartum clinics. Warfarin sodium was continued, and the patient was followed with prothrombin time, determinations in the medical clinic. Eight weeks after discharge, the patient was readmitted to the gynecologic ward because of intense lower abdominal pain. Pelvic examination revealed the cervix to be displaced behind the pubic symphysis. The uterus and adnexa were not well delineated because of moderate abdominal distention. The pouch of Douglas was bulging. Culdocentesis retrieved 20 ml. of unclotted blood, and diagnosis of hemoperitoneum was made. An exploratory laparotomy was performed and revealed 500 ml. of free blood in the abdominal cavity. The left ovary, tube, and uterus were normal. The right ovary was occupied by a 6 by 7 by 8 cm. ruptured hemorrhagic cyst, and right ovarian cystectomy was performed. The pathologic diagnosis of the submitted specimen was hemorrhagic corpus luteum cyst.

Comment

The incidence of hemorrhagic complications following anticoagulant therapy is approximately 10 per cent if minor and major complications are included.[3] Nevertheless, these agents are indispensable in the management and prevention of thromboembolic phenomena. In a study of 1,626 patients, Askey[3] showed that intracranial and gastrointestinal hemorrhage are the most serious complications as measured by deaths and account for over 90 per cent of serious bleeding episodes following anticoagulant therapy. Corpus luteum cyst hemorrhage may occur with anticoagulant therapy; however, massive hemoperitoneum is rare.[4, 5] Life-threatening intraperitoneal hemorrhage secondary to ruptured ovarian hemorrhagic cyst due to anticoagulation, which occurred in the cases presented, is not uncommon. Premenopausal patients on long-term anticoagulant therapy are at a risk of developing ovarian hemorrhagic cysts. The treating physicians must be aware of the possibility of hemoperitoneum in these patients. Pelvic examination should be performed frequently to determine the presence of ovarian enlargement. In the obese patient, pulsed ultrasound may be of assistance in determining the presence of ovarian cysts. Early termination of anticoagulant therapy should be considered in patients with enlarging ovarian masses. Diagnostic culdocentesis should be performed in patients with abdominal pain, and laparotomy is indicated when free blood is found in the pouch of Douglas. Protamine sulfate and vitamin K and/or fresh blood will reverse the effect of warfarin sodium.

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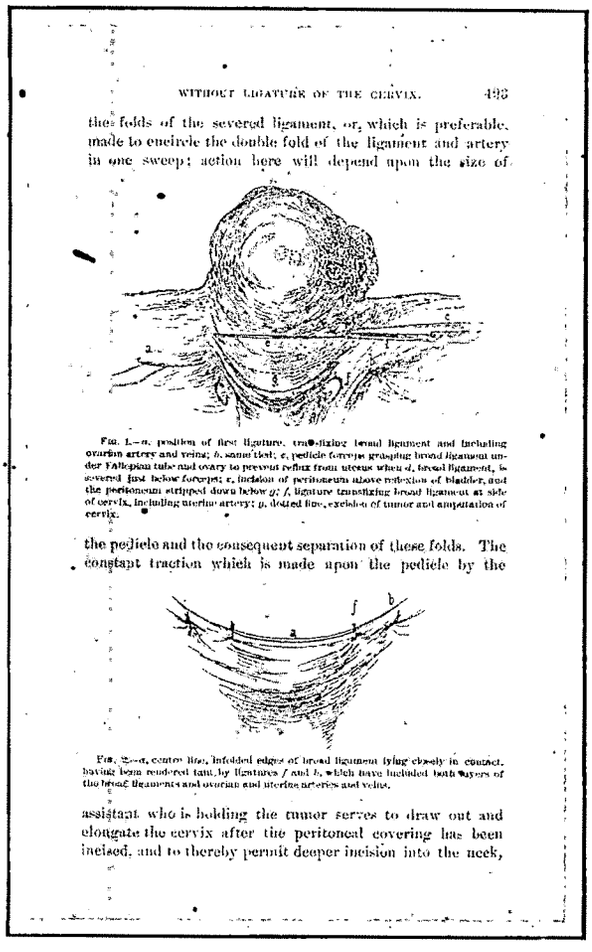
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Classic pages in
OBSTETRICS AND GYNECOLOGY
Lawrence D. Longo, M.D.

Supravaginal hysterectomy without ligature of the cervix, in operation for uterine fibroids: A new method

BENJAMIN FRANKLIN BAER

American Journal of Obstetrics and Diseases of Women, vol. 26, pp. 489-504, 1892



WITHOUT LIGATURE OF THE CERVIX. 493

thes folds of the severed ligament, or, which is preferable, made to encircle the double fold of the ligament and artery in one sweep; action here will depend upon the size of

FIG. 1.—*a*, position of first ligature, embracing broad ligament and including ovarian artery and veins; *b*, same tied; *c*, pedicle forceps grasping broad ligament under Fallopian tube and ovary to prevent reflux from uterus when *d*, broad ligament, is severed just below forceps; *e*, incision of peritoneum above reflexion of bladder, and the peritoneum stripped down below *g*; *f*, ligature transfixing broad ligament at side of cervix, including uterine artery; *g*, dotted line, excision of tumor and amputation of cervix.

the pedicle and the consequent separation of these folds. The constant traction which is made upon the pedicle by the

FIG. 2.—*a*, centre line, infolded edges of broad ligament lying closely in contact, having been rendered taut by ligatures *f* and *b*, which have included both layers of the broad ligaments and ovarian and uterine arteries and veins.

assistant who is holding the tumor serves to draw out and elongate the cervix after the peritoneal covering has been incised, and to thereby permit deeper incision into the neck,

During the latter part of the nineteenth century, supravaginal hysterectomy attracted considerable attention because of the problems of hemorrhage and sloughing that often followed en masse ligation of the cervical stump. Baer, of Philadelphia, improved upon the technique of subtotal hysterectomy by ligating the ovarian vessels and then ligating the uterine vessels while elevating the uterus. The cervical stump was then dropped below the peritoneum without suture in it or a ligature constricting it, and the peritoneum was closed over the stump (see left). Baer presented ten cases with one death, presumably from the anesthesia. He enumerated the rationale for the method as: "1st. It is secure against hemorrhage, because the blood vessels are ligated outside of the muscular tissue of the cervix; and against sloughing, because these tissues are entirely free from a constricting ligature or suture. 2d. It removes all of the supravaginal tissue, but does not open the vagina, thereby permitting the vaginal portion of the cervix to remain attached and in situ, to maintain its position as the keystone of the arch, and to preserve the strength and anatomical shape of the lower portion of the abdominal cavity. 3d. The raw end of the stump is retracted deeply in the pelvis, where it is surrounded and covered by the other raw surfaces and the peritoneal flaps. . . . 4th. The pelvic cavity, when the operation is finished, has its natural lining of peritoneum, and is free from the danger of contamination from a sloughing pedicle, open vagina, or a drainage tube. 5th. The parts are left in their natural relation with one another. . . . 6th. It is not unduly mutilating, requires the minimum number of ligatures, and is applicable to the worst cases. 7th. Both the operation itself and the convalescence are shorter than by any other method. 8th. The unpleasant and often painful and dangerous sequelae, as hernia, fistula, etc., are absent." Baer also pioneered in advocating closure of the abdomen without drainage in cases of pelvic abscess and wrote on many aspects of gynecologic surgery.

CURRENT DEVELOPMENTS

Puerperal infection in the antibiotic era

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Developments in etiology and treatment of puerperal infection in the last 20 years are reviewed. Emphasis is placed upon predisposing factors, microbiology and antibiotics, unusual sources of severe infection, special complications, and prophylactic antibiotics.

THE CONTROL of obstetric infection has been one of the gratifying successes of the antibiotic era. Even in the mid-1970's, however, puerperal infection continues to cause continuing morbidity and deaths.

From the early 1930's until the 1950's, puerperal infection decreased in both frequency and severity. In 1936, sulfonamides were first used in treating puerperal sepsis.[1, 2] In the next ten years, penicillin and streptomycin were introduced into general use. However, even prior to the use of these antibiotics, the β-hemolytic streptococcus, the organism responsible for epidemics of puerperal sepsis, appeared to be undergoing a spontaneous decrease in virulence.[2] In addition, blood transfusions had become readily available and, with the use of oxytocic agents and cesarean section, the incidence of prolonged labors and traumatic deliveries decreased markedly. Since the early 1950's, there has been little change in the morbidity rate[3–6] and perhaps even an increased maternal mortality rate[7] from infection despite the development of even more potent antibiotics.

Epidemiology

Incidence. Estimates of the incidence of postpartum infection are difficult to assess. Many report the incidence of "standard puerperal morbidity," which is defined by the U. S. Joint Committee on Maternal Welfare as "a temperature of 100.4° F. (38.0° C.), the temperature to occur in any two of the first 10 days postpartum, exclusive of the first 24 hours, and to be taken by mouth by a standard technique at least four times daily." Because of early patient discharge practices, the full criteria of the original definition can no longer be applied. In addition, it has been noted that many patients with infection respond to antibiotics so quickly that they do not meet the temperature criteria for standard morbidity. Thus, in one group of patients with endometritis, only 56 per cent had standard morbidity.[8]

Others use different criteria for morbidity, ranging from a temperature of greater than 99.4° F. on any occasion[9] to a temperature of greater than 101° F. after the second postpartum day.[136] Still others refer to the clinical signs and symptoms of infection or the positive growth of a significant number of pathogenic organisms.[10] Finally, in some institutions, antibiotics have been administered routinely for such reasons as breech deliveries, "major" episiotomy incisions,[11] cesarean section, or gross fecal contamination of the vagina or the perineum.[3] When antibiotics are given on such grounds for a number of days, these may be therapeutic and might be responsible for a deceptively low morbidity rate.

Low-grade temperature elevations are very common in the postpartum period and often resolve spontaneously. Calman and Gibson[9] reported that over one third of puerperal patients had at least one temperature greater than 99.4° F. Of these fevers, over one

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half were transient and resolved spontaneously. The etiologies of such fevers include dehydration and reaction to an infusion of fetal proteins. In most reports, genital tract infection is the commonest source of postpartum fever,[3, 9] but others report urinary tract infection to be the most common.[8, 12] Among other sources are mastitis, breast abscess, dehydration, and respiratory infection.

The critical observer must consider all of these factors in assessing obstetric morbidity reports. It is clear, however, that fevers in the puerperium have decreased in most centers to 15 to 50 per cent of the incidence in 1930.[3–5] In one report in which the birth canal infection rate did not change, the incidence was already at the remarkably low rate of 2 to 3 per cent in 1928.[6]

The decreasing severity of infection is witnessed by a shortening duration of fever[6] and by marked reduction in deaths from infection. In England and Wales, the maternal mortality rate from puerperal infection fell from 1.5 per 1,000 births in the early 1930's to 0.006 per 1,000 births in 1963.[6]

At present, the rate of postpartum infection may be estimated to be from 1[13] to 8 per cent[5, 10] with occasional reports of morbidity rates as high as 30 per cent.[12]

A small number of retrospective reports have focused on cases of maternal death from infection. In Navy Hospitals, from 1962-6,[14] there were 51 deaths among 170,012 births. Infection was directly or indirectly a factor in 50 per cent of the obstetric deaths and only three were associated with septic abortion. In the United States, in 1963, infection was the second most common cause of maternal deaths, accounting for 25 per cent.[14] Approximately half were associated with term or near-term pregnancy and half were associated with abortion. In two reports, Stevenson and colleagues[15, 16] detailed 91 cases of maternal deaths from puerperal sepsis in Michigan in 1950-1966. Forty-seven were associated with cesarean section and 44 followed vaginal delivery. In 5 of the last 6 years of the report, infection was the leading cause of maternal death, and it was concluded that the deaths were preventable in over two thirds of the cases, with both patient and medical personnel sharing responsibility equally. Patient responsibility consisted primarily of delay in seeking medical care (for example, after prolonged rupture of the membranes); physician and hospital responsibility consisted of inadequate evaluation of symptoms, incorrect diagnosis, failure to hospitalize earlier, and inadequate institution of antibiotic therapy.

Epidemics. Lethal epidemics of β-hemolytic streptococcal infection occurred as late as 1927 when, at Sloane Hospital in New York, 24 mothers were infected and 9 succumbed. In the absence of antibiotics, the epidemic was brought under control only by closing the hospital for 10 days.

Six outbreaks of streptococcal infection have been reported since 1953, including five since 1965.[17–22] These outbreaks have all involved Group A β-hemolytic streptocci (usually one type) and involved from two to 20 mothers, none to 11 newborn infants, and many of the hospital staff. Although there were no maternal or neonatal deaths, a number of the patients were severely ill. There are no reported epidemics of puerperal infection due to organisms other than Group A streptococci.

Genital tract infection

Predisposing factors. Five factors appear to be major predisposing factors to postpartum infectious morbidity and death. These are: cesarean section, "prolonged" labor and rupture of the membranes (ROM), trauma at delivery, anemia, and low socioeconomic status. Factors with possible roles are: vaginal (as opposed to rectal) examinations, manual removal of the placenta, episiotomy, forceps deliveries, and intrauterine labor monitoring.

Cesarean section. This is the most important factor predisposing to both the frequency and severity of postpartum infection. Goplerud and White[5] found that 17 per cent of their infected patients had cesarean section, whereas the overall occurrence of cesarean section was only 3 per cent. Humphrey[10] found that 41 per cent of his infected patients had abdominal deliveries when the over-all cesarean section rate was 5.6 per cent ($P < 0.2$ per cent). Sweet and Ledger[8] reported the rate of endometritis following cesarean section to be increased sevenfold over the rate following vaginal delivery at Wayne County Hospital. Recent studies employing prophylactic antibiotics have been stimulated by baseline infection rates of between 51 and 64 per cent.[143–147] It has already been noted that patients having cesarean sections comprise over half of the maternal deaths from infection in the Michigan study.[15]

While abdominal delivery in general increases morbidity, classical cesarean section specifically places the parturient at even further risk. Of the 47 deaths following section in Michigan, 26 were associated with classical operations and 19 with low-segment operations, which are performed much more commonly.[15] Over 36 years at the University of Iowa, there were 3,600 cesarean sections and six deaths from infection. Four occurred after classical cesarean section and two

Table I. Perinatal deaths and maternal morbidity associated with PROM

Ref No.	*Year*	*No. of patients*	*Population*	*Gestational ages*	*Perinatal deaths*	*Maternal morbidity*
25	1961	515	Private Hospital	20-36 wks.	PROM > 48 hr. 27.7%* PROM < 48 hr. 12.3%*	7.8% for PROM 1.8% for no PROM
30	1963	1,896	Naval Hospital	All	1% for all with PROM < 14 days	7.3% for PROM, no antib. 3.5% for PROM, w/antib. 6.4% no PROM, no antib.
26	1964	1,788	Indigent	All	PROM < 24 hr. 2.3% PROM = 24-48 hr. 4.5% PROM > 48 hr. 8.2%†	10.6% postpartum fever if PROM > 48 hr. 8.6% antepartum fever if PROM > 48 hr.
28	1965	473	½ private, ½ indigent	All	No PROM, 4.1% All PROM, 6.1% *PROM* > 24 hr. 11.6%‡	28% w/PROM > 24 hr.
31	1967	415	Private	All	0-2.5% ≥ 34 wk. for all PROM	2.6% endometritis
29	1970	450	UCLA	All	w/chorioamnionitis, 16% over all 31% 1,000-2,499 Gm.	26.4% chorioamnionitis if PROM > 24 hr.

*Fetuses 1,500 to 2,500 Gm. only.
†If mother developed fever antepartum, PNM was 24.3%.
‡If mother developed fever antepartum, PNM was 50%.

after cesarean hysterectomy. All three maternal deaths from 1941 to 1961 were associated with classical cesarean section. There were no deaths from infection in 935 low-segment cesarean sections.[5]

The explanation for increased infection with cesarean section presumably lies in increased intrauterine manipulation, the presence of foreign body (suture material), necrosis of tissue at the suture line, hematoma formation, and wound infection. The additional risk of classical cesarean section arises from the inability to cover the uterine incision with the vesicouterine peritoneum and thus to restrict the infection.

Prolonged labor and prolonged rupture of the membranes. Patients enduring long labors appear to be predisposed to postpartum infection, but there has been little agreement on the definition of "prolonged" labor. Utilizing 10 hours as the differentiating time, Fara and co-workers[23] found no significant difference in infection rates, but Humphrey[10] used a duration of more than 18 hours of regular contractions to define prolonged labor and reported a highly significant increase in the prolonged labor group ($P < 0.01$). Others have noted that patients with labors lasting over 30 hours were found twice as frequently in the infected group as in the uninfected group.[5]

Perhaps more important than the length of labor *per se* is the length of rupture of membranes (ROM). Among 44 infectious maternal deaths in Michigan, there were only 10 labors longer than 10 hours, but the average duration of ROM was 90 hours.[16] In 1967, Webb[24] reviewed 54 cases of maternal death associated with premature rupture of membranes (PROM) in California; 38 died of sepsis and eight others had sepsis as a contributing factor in death.

Nonetheless, reports on the management of the patient with PROM have reached conflicting conclusions. The two parameters of greatest importance are perinatal mortality (PNM) and maternal infection. Variables such as the stage of gestation studied, use of antibiotics, and socioeconomic status of the patient population may explain the difference in the conclusions (see Table I).

In 1961, Breese[25] reported on 10 years' experience involving premature pregnancies with premature spontaneous rupture of the membranes at a private hospital in Oregon. In 515 patients (infant weights, 501 to 2,500 grams), 50 per cent had latent phases of labor longer than 48 hours, and 24 per cent more than 7 days. For infants between 1,501 and 2,500 grams, PNM with a latent phase less than 48 hours was 12.3 per cent and PNM with a latent phase greater than 48 hours was 27.7 per cent. Maternal morbidity in general was 1.8 per cent, whereas in patients with PROM of any length it was 7.8 per cent. Mothers with latent phases of less than 48 hours had a morbidity of 7.3 per cent and those with longer latent phases had a morbidity of 8.3 per cent. Many patients in the latter group received

antibiotics. (A full discussion of antibiotics and PROM appears in the discussion of developments in therapy.) With the use of antibiotics (usually penicillin and/or streptomycin) an improvement in PNM was noted.

Studying indigent patients in Chicago, Burchell[26] observed a doubling of PNM after a latent period of 24 hours and a redoubling (to 8.1 per cent) after a latent period of 48 hours. If the mother became febrile before delivery, the PNM increased eightfold to 24.3 per cent. In terms of maternal infection, a latent period of greater than 24 hours was associated with a fourfold increase of maternal antepartum fever when compared with latent period of less than 24 hours. Patients with a latent phase of more than 48 hours had a higher morbidity rate (10.6 per cent) as manifested by postpartum fevers than those with a latent phase of 24 hours (7.9 per cent) or with a latent phase of 24 to 48 hours (6.9 per cent). The use of antibiotics (type not specified) appeared to decrease PNM and maternal intra- and postpartum fever.

Russell and Anderson[27] reported a decreased incidence of amnionitis, decreased PNM, and decreased maternal mortality rates in service patients by delivering all patients more than 36 weeks pregnant within 18 to 24 hours of the *confirmation* of the diagnosis of PROM. Induction was successful in 95 per cent.

In a population that was approximately half private and half ward, Lanier and associates[28] noted that PROM greater than 24 hours was associated with a threefold increase in the perinatal mortality rate to 11.6 per cent. Maternal fever occurring prior to delivery was associated with a striking PNM of 50 per cent, and maternal infection occurred in 28 per cent of patients with ROM more than 24 hours before delivery. The average duration of ROM was 55 hours—median 36 hours—but the average duration of time in the hospital prior to delivery was only 20 hours. Thus, despite aggressiveness with induction, results were still poor. Analyzing various factors, the difference between those who became infected and those who did not appeared to be "race with its attendant indigency." Similar experience has been reported from the Medical College of Georgia.[28] In 1970, Gunn and co-authors[29] published a 10 year review of the experience with PROM at UCLA and an extensive review of the literature. Among their patients, amnionitis developed in 9.1 per cent of all patients with PROM (defined as ROM at any time prior to onset of labor). In patients with PROM over 24 hours, amnionitis occurred in 26.4 per cent (121 of 450) among all birth weight groups and in 31.2 per cent (43 of 138) of premature infants (1,000 to 2,499 grams). The over-all PNM in patients with chorioamnionitis was 16 per cent, whereas in the premature group the PNM was 24 per cent. In this study, no data could be obtained on postpartum maternal morbidity in patients with PROM. It was proposed that after 12 to 18 hours of PROM, labor should be induced to achieve delivery within 24 hours for fetuses with an estimated weight of over 2,000 grams. In cases of an estimated fetal weight of less than 2,000 grams, an "expectant" therapy was advised, with hygienic perineal care, relative bed rest, frequent temperature recordings, and no examinations after an initial sterile speculum examination to confirm ROM.

Other studies, however, indicate more benign outcomes. In a multihospital Navy study,[30] tetracycline was given to patients with PROM until spontaneous labor ensued and for 3 days postpartum. Patients in whom labor was induced when PROM was diagnosed were excluded. Standard morbidity, in general, and endometritis, in particular, were decreased even compared to patients without PROM. Although there was no decrease in PNM with the use of tetracycline, the over-all PNM (for mature infants) averaged only 10 per 1,000 births. At a Kaiser-Permanente Hospital, Sachs and Baker[31] noted no increase in PNM nor increase in maternal infection when they awaited spontaneous labor without the use of antibiotics. Approximately 25 per cent of their patients had latent phases of greater than 48 hours.

Despite conflicting evidence, it appears to be most reasonable to deliver within approximately 24 hours of definite ROM in pregnancies greater than 36 weeks. For smaller fetuses, management should be based upon the relative risks of infection and of prematurity (mainly respiratory distress syndrome [RDS]) at a particular hospital. Recent preliminary data suggest that it may be advantageous to wait at least 24 hours before effecting delivery in those cases with ROM at less than 36 weeks. Yoon and Harper[32] found a decreased incidence of RDS in infants with ROM over 24 hours, and Richardson and co-workers[33] reported that the lecithin-sphingomyelin ratio in the amniotic fluid may mature at an accelerated rate after ROM. It is not clear, however, whether the over-all PNM (including causes other than RDS) is decreased by waiting more than 24 hours or whether there is a "critical" duration of ROM after which there is no appreciable decrease in the incidence of RDS. At this time, the evidence does not justify sweeping changes in management but determinations of L/S ratios in individual cases may be helpful. Further prospective studies will be of major importance.

Trauma. Traumatic deliveries are believed to predispose the patient to infection by providing a

weakened host and a favorable environment for bacterial reproduction. Yet, only a small percentage of infected women sustain major delivery trauma.[5]

Socioeconomic status. Most reports indicate that indigent patients (independent of race) have higher morbidity rates than middle-class patients,[4, 11, 40] especially in relation to PROM and cesarean section.[8] Many factors have been assessed to explain this observation. Walsh and colleagues[34] found a significantly higher incidence of pathogenic organisms in 300 lower genital tract cultures of 28 pregnant ward patients than in 15 pregnant private patients. Patients of lower socioeconomic status have unfortunately also been subject to less prenatal care, poorer hygiene, poorer nutrition, and higher incidence of anemia. In labor, they are examined more frequently and delivered by the least experienced personnel.

Anemia. Also well established is the association between anemia and puerperal infection.[5, 10, 35, 36] It is not clear, however, whether infected patients with anemia respond more promptly after receiving a transfusion.

Vaginal examination and the number of examinations. The concern that once prevailed over performing vaginal examinations in labor has been abolished by both retrospective[23] and prospective clinical and microbiologic studies.[37, 38] At Cook County Hospital,[23] no difference was found in morbidity between the patients having one vaginal examination and those having many.

Manual removal. Thomas[39] cited a maternal mortality rate of 3.3 per cent and a maternal morbidity of 32 per cent associated with manual removal prior to 1945. Many of these patients, however, may have had some intercurrent condition such as severe hemorrhage since the experience recently has been much less impressive. In 1963, no increase in infection was found in 921 patients having manual removal. Jones and co-workers[40] in 1966 reported a decreased rate of infection by routinely performing manual removal. Higher morbidity rates have been reported in two small series.[41, 42]

Despite the evidence that manual removal does not increase puerperal infection, it is still not widely advocated for routine use. Charles and Klein[35] favored careful inspection; Hellman and Pritchard[36] advised removal only after thirty minutes.

Episiotomy and forceps deliveries. Two retrospective studies[10, 23] indicate that the performance of an episiotomy with or without forceps delivery significantly increases the incidence of puerperal infection. Further, the incidence of operative deliveries was reported to be twice as high in infected as in uninfected patients,[5] and the incidence of positive blood cultures following forceps deliveries was higher than that following spontaneous deliveries.[3] Patients having either episiotomies or forceps deliveries may also have had more difficult labors. While interference must be kept at a minimum, the advantages of a episiotomy incision over a laceration, and of indicated or even elective low forceps, usually outweigh the increase in infection.

Intrauterine monitoring. With the increased use of intrauterine monitoring, concern has arisen with regard to increased maternal infection. Schurz and co-workers[43] found no increase in infection in 110 intensively monitored patients, but patients with ROM greater than 6 hours were not monitored. Penicillin was added to the flushing solution, moreover.

In a review of 1,150 internally monitored patients, Chan and co-workers[44] found only six cases of endometritis. Sixty of 66 patients with standard febrile morbidity were felt to have sources of fever other than endometritis. The incidence of standard pyrexia in electively monitored patients was no higher than in nonmonitored patients (1 to 2 per cent). In the patients with indicated monitoring the incidence of standard pyrexia was 7 per cent (10 of 150). Of these, only one patient was thought to have endometritis. Eight of these 10 pyrexic patients had cesarean sections; however, since the total number of cesarean sections is not given, morbidity data in this select group cannot be determined. No data on the average duration of ROM and of monitoring are presented. Thus, the incidence of "endometritis"—a surprisingly low 0.5 per cent—in this population must be viewed with caution, especially when compared to the standard pyrexia of 5.0 per cent. In a similar study, Wiechetek and co-authors[45] analyzed 148 internally monitored patients over 3 years. No significant difference in the incidence of morbidity or endometritis was found between the monitored and control groups. Cesarean section was associated with a morbidity of 35 per cent for both monitored and unmonitored patients while for vaginal delivery the morbidity of both groups was 6 per cent. Also, no correlation was found between incidence of endometritis and duration of monitoring up to 5 hours. On the other hand, Ledger and associates[46] have found that internal monitoring was associated with a small, but highly significant ($p < 0.001$), increase in the incidence of postpartum bacteremia from 0.5 to 1.4 per cent.

In a prospective evaluation of 86 monitored patients, Larsen and colleagues[47] reported a progressive increase in the incidence of intrauterine infection with the duration of monitoring. Of patients monitored for

1 to 4 hours, 25 per cent were morbid. Of those monitored for 4 to 8 and > 8 hours, respectively, 50 and 71 per cent were morbid. The presence of one or more polymorphonuclear leukocytes per high-power field in the amniotic fluid in 16 patients was associated with infection in all. In the absence of PMN's a number of patients still became morbid. In this report, however, morbidity was defined as only one temperature reading *at any time* greater than 100.4° F. Perhaps in part because of this definition, the incidence of infection was unusually high. Presence of bacteria in the culture was of no significance.

Microbiology of puerperal infection.

Difficulties in making the diagnosis. Making the specific diagnosis of puerperal genital tract infection is a difficult clinical and microbiologic task.

1. Many studies have demonstrated that the normal flora of the vagina and cervix consists of many species of organisms.[34, 48–50] Among the organisms usually considered nonpathogens are: lactobacilli (Doderlein's bacillus), diphtheroids, coagulase-negative staphylococci, and micrococcus. Organisms frequently found, but still considered potential pathogens, are: all aerobic hemolytic and nonhemolytic streptococci, anaerobic cocci, coagulase-positive staphylococci, gonococci, clostridia, *E. coli,* Klebsiella, Enterobacter, Proteus, Pseudomonas, Bacteroides, and Mycoplasmas and T-form mycoplasmas.

2. As noted, low-grade, transient fevers are common and appear to be of questionable significance.[9]

3. The puerperal patient with a sustained fever often has few specific signs and symptoms.

4. Though urinary tract infections are easily documented with a positive culture, it is often impossible to be certain they are the cause of the fever. Especially in the absence of urinary tract symptoms, one cannot attribute a fever to urinary tract infection solely on the basis of a positive culture.

5. Even when there is good clinical evidence of genital tract infection, the bacteriology report may not be confirmatory. Detailed studies have shown remarkable similarities between the flora of the "infected" and "uninfected" uteri.[51–53] Moreover, unless anaerobic techniques are used, there will often be a report of "no growth" or a poor correlation between the culture results and gram stains and positive blood cultures.[8, 49]

6. High vaginal or even cervical cultures are inadequate in determining the flora in the endometrium. Such a practice is similar to culturing the throat instead of the sputum, in a case of pneumonia. Cultures should be done of the endometrium because Calman and Gibson[52] showed the upper vagina to contain more organisms than the endometrium.

7. Gram stains should be done and types and relative numbers of bacteria noted. Yet, often only occasional organisms are seen. The presence of intracellular organisms is very suggestive of infection, but such forms may be difficult to detect.

8. Although recovery of organisms from the blood is very helpful, positive blood cultures are seen in only about 8 per cent of cases.[3, 8]

The present picture. Table II lists the results of a number of studies indicating the source of the culture and culturing techniques used. The most common organisms causing postpartum infection have been reported to be *E.coli*[8, 56] and anaerobic streptococci.[3, 52, 53] A suggestion of a changing microbiologic picture comes from cases of fatal sepsis at Queen Charlotte's Hospital in England. In 1933 to 1935, there were 86 maternal deaths from infection, 75 per cent from hemolytic streptococci, 10 per cent from anaerobic streptococci, 5 per cent from *S. aureus*, and 2 per cent from *E. coli.* From 1946 to 1968, there were only four deaths, two with *Cl. welchii*, one with Pseudomonas, and one with Proteus.[6] From three retrospective studies of maternal deaths from infection, [14–16] the organism cultured most often was *E. coli.* The gram-positive organisms were cultured next most frequently, but in far fewer cases.

CHARACTERISTICS OF COMMON PATHOGENS.

Aerobic streptococci: The streptococci are classified by a number of systems, resulting in a confusing nomenclature. The aerobic streptococci may be classified by their hemolytic properties on blood agar plates. Organisms showing partial or green hemolysis are called α-streptococci (also known as *S. viridans*); those showing complete or clear hemolysis are β-streptococci; those without hemolysis are γ-streptocci. The hemolytic properties of a species may, however, be variable.[63] Many streptococci may also be classified into serologic (Lancefield) groups by a precipitin reaction. Groups A, B, and D are currently known to be pathogenic. Rarely, other groups may cause pelvic sepsis.[63]

1. Group A β-hemolytic streptococci *(S. pyogenes).* The organism is rarely found in the cervix or vagina of antepartum patients[34, 38, 54] and occasionally in the postpartum pelvis in nonepidemic situations.[8, 53] The clinical picture of infection with this organism is characteristic.[55, 115] The initial fever spike is high, occurs early, and is associated with chills and prostration. Tenderness over the uterus is diffuse and may extend to the parametria. Early peritoneal signs and ileus may be present. The lochia is scanty, is not foul, and contains many gram-positive cocci in chains on Gram stain. Group A β-streptococci are sensitive to

Table II. Pathogenic organisms cultured from "infected" puerperal patients

Date and location	*Ref. No.*	*No. of patients*	*Source of culture*	*Anaerobic cult used*	*Most common organism*	*Second most common*	*Third most common*
1947, Chicago	51	45	Endometrical	Yes	γ-streptococci 40%	Bacteroides sp. 37.8%	Ana strep. 33%
1953, London	52	141	High vaginal	Yes	Ana strep. 34%	*S. aureus* 16.5%	Aer. non-hemol. strep. 13.5%
1959, N.Y.C.	3	?	"Lochia"	Yes	Ana strep. 68%	Aer. non-hem. strep. 24%	*E. coli* 22%
1973, Iowa	56	600	?	Yes	*E. col.* 148 isol.	α-strep. 84 isol.	β-strep. 73 isol.
1973, Michigan	8		? Cervix	Yes	*E. coli* 39 isol.	Ana. strep 32 isol.	Bacteroides 31 isol.
	Wayne Cnty	143	? Endometrium				
	UMMC	101	? Cervix	Yes	*E. coli* 57 isol.	*S. viridans* 26 isol.	*S. faecalis* 19 isol.
			? Endometrium				
1974, Phila.	53	27	Endometrial	Yes	Ana strep 37%	Ana staph. 33%	Bacteroides 29%

penicillin. In the acutely ill, penicillin-allergic patient, erthromycin or cephalosporin may be substituted. Response to appropriate antibiotic therapy is usually prompt.

Since epidemics of Group A β-hemolytic streptococci disease usually are presumed to be exogenous in origin from the nasopharynx or skin lesion of a mother, infant, or hospital staff member, preventative procedures should be employed routinely. Nasopharyngeal or skin cultures on obstetric personnel and patients with upper respiratory infections or suspicious skin lesions should be obtained, and patients with suspected β-streptococcal puerperal infection should be isolated. Personnel with a positive culture should not work with labor or maternity patients until antibiotic therapy has been instituted and their culture has become negative. Close liaison with the microbiology laboratory for immediate report of any Group A β-streptococci from febrile patients should be established. If the organism is found, both patient and neonate should be treated and isolated.

Once two or more cases have been reported in a short interval, an epidemic can be presumed to be in existence. In these instances, additional measures are in order. A health department epidemiologist should be requested to advise and aid in identifying the possible source. Infected patients should be isolated and treated. In most epidemics, still further measures have been necessary, since recognition of the epidemic may have been delayed. All personnel involved in the care of maternity patients should be screened for Group A β-streptococci with nasopharyngeal and skin lesion cultures. If culture is positive, carriers should be removed from duties with maternity patients and treated with oral antibiotics for 10 days. Skin lesions should be recultured on the third day, and throats on the seventh day. These personnel should not return to regular maternity duty until they are culture negative. A system of cohort nurseries may be instituted, and elective inductions, circumcisions, and perhaps even elective surgery may have to be cancelled.

When the epidemic is widespread,[20, 22] prophylactic penicillin or sulfa drugs may need to be given to all patients (mothers and neonates) and all staff.

2. Group B β-hemolytic streptococci (*S. mastitidis, S. agalactiae*). This organism has generated interest in the past few years because of reports of its increasing role in puerperal and neonatal sepsis. Group B streptococci are found in 5 to 14 per cent of vaginal or cervical cultures of pregnant women in the United States and Europe.[54, 56–58] It is impossible to determine whether the carriage rate has increased because older studies did not type β-hemolytic streptococci. Undoubtedly, though, Group B and other non–group A species have become far more important than Group A. In contrast to Group A infections, Group B infections occur endemically from the mother's endogenous flora. Group B streptococci generally are reported to be exquisitely sensitive to penicillin (drug of choice) and also to ampicillin and erthromycin.[59] Clinical response in the mother is usuably rapid, though severe infections including meningitis and death have been reported.[58, 60]

For the neonate, Group B streptococci are particularly pathogenic and are the most common gram-positive organism causing neonatal sepsis.[60] The neonate who develops Group B streptococcal sepsis is colonized most probably during labor or at birth. Since only a small percentage of pregnant women carry this organism, it has been proposed that such carriers be

treated antepartum.[58, 61, 62] The potential control of Group B streptococcal sepsis is difficult.[61] First, it is not clear what percentage develop sepsis. Franciosi and associates[61] found in culturing the throats of 36 neonates of Group B streptococci–positive mothers that only five were positive. Second, a number of infants colonized by Group B streptococci are born of Group B streptococci–negative mothers. Third, labor floor and nursery personnel may be carriers of Group B streptococci. Fourth, the male urethra harbors Group B streptococci, which may reinfect the female. Fifth, on occasion Group B streptococci are extremely difficult to eradicate from the vagina. Finally, there appears to be a wide variation in the incidence of Group B streptococcal neonatal sepsis. A rational approach to the treatment of either mother or neonate must await further data.

3. Other aerobic streptococci. The α-streptococci are commonly found in the pelvis, but are usually of low pathogenicity and sensitive to penicillin. Of special importance is another species, enterococcus (which includes *S. faecalis* and other less common species) which may be either α- or γ-hemolytic. Most enterococci are Group D, but not all Group D streptococci are enterococci. Characteristically resistant to penicillin alone, it is usually sensitive to ampicillin or a penicillin plus an aminoglycoside (streptomycin, kanamycin, or gentamicin).[64] Combinations of a cephalosporin or clindamycin plus an aminoglycoside provide inadequate coverage against enterococci. In the penicillin-allergic patient, vanomycin is appropriate.

Aerobic staphylococci *(S. epidermidis and S. aureus):* Aerobic species possessing a coagulase enzyme may be pathogenic. *S. aureus* has been found in 5 to 17 per cent of genital tracts of various populations, and *S. epidermidis* still more commonly.[3, 8, 10, 34, 48, 51, 53] These organisms may cause endometritis, pelvic and vulvar abscesses, breast abscess, or septicemia with metastatic abcess formation. Staphylococcal epidemics in nurseries occur on occasion, usually from the nasopharynx or skin of a hospital worker, but have not been reported among mothers. The ability of some *S. aureus* to produce penicillinase makes it resistant to penicillin and ampicillin. Other antibiotics such as oxacillin, methicillin, or cloxacillin and the cephalosporins are generally effective.

Aerobic gram-negative bacilli: The increasing importance of these organisms has already been emphasized. Clinically, these infections vary from mild to fulminant. Indeed, these organisms are the most commonly found in puerperal bacteremia[46, 139, 141] and in puerperal septic shock.[114, 115] The lochia is nonfoul in pure infections. In the laboratory, these organisms grow rapidly and are readily distinguished from each other by biochemical tests. Antibiotic resistance may develop and may even be transmitted from one species to another by extrachromosomal genetic factors (R factors).[65] Therefore, specific antibiotic testing is mandatory.

1. *Escherichia coli.* Among the most numerous species in the human gastrointestinal tract, *E. coli* has been found in the vagina or cervix of from 5 to 38 per cent of antepartum patients.[31, 34, 57, 50] Responsible for a wide variety of human infections, it has been reported to be the most common organism isolated in puerperal infection, especially in bacteremia and septic shock. In the laboratory it forms gas, and its colonies appear as a dark lactose fermenters on MacConkey agar. Its sensitivity to antibiotics varies from hospital to hospital and perhaps from service to service within a hospital, but kanamycin, gentamicin, and chloramphenicol are generally effective against over 95 per cent of species.[65] Cephalosporins are active against 80 to 93 per cent, and ampicillin and tetracycline against perhaps 80 per cent.[65]

2. Klebsiella-Enterobacter. These organisms have been found in some reports in 5 per cent of antepartum lower genital tracts,[46, 50] but other studies have failed to recover them.[34, 37] In postpartum urinary and genital infections they play an occasional role, far less important than *E. coli* The characteristic mucoid appearance of the colonies gives an early, important clue in their laboratory identification. Gentamicin is nearly 100 per cent effective, and kanamycin and chloramphenicol are effective against 85 to 95 per cent. Cephalosporins are highly effective against Klebsiella species, but not against Enterobacter; ampicillin is essentially useless against either.[65]

3. Proteus. These species are reported in no more than 2 to 4 per cent of antepartum patients.[34, 50] Two distinctly different groups, with different biochemical and sensitivity patterns, are found. The indole-negative species, *Proteus mirabilis*, is highly sensitive to kanamycin and gentamicin (>95 per cent) and only slightly less sensitive to ampicillin and cephalosporins (85 to 90 per cent). Indole-positive species, *P. vulgaris* and *P. morganii*, are also highly sensitive to kanamycin and gentamicin, but much less so to ampicillin and cephalosporins (~ 40 per cent). Tetracycline is of little value against either.[65]

4. Pseudomonas. An opportunistic pathogen in severe nosocomial infections, Pseudomonas is only rarely found in the antepartum cervix.[50] A characteristic, pleasant, grape-like odor is given off by its colonies. Antibiotics of choice in treating Pseudomonas infection

are gentamicin, carbenicillin, or colistin.[65] In life-threatening infections, antimicrobial combinations such as gentamicin and carbenicillin may be used because of synergism.

Anaerobic gram-positive organisms: The strictly anaerobic streptococci (peptostreptococci) continue to play a major role in puerperal infection.[8, 34, 53, 66] Infections due to peptostreptococci characteristically occur in the presence of traumatized or devitalized tissue and give a foul lochia. Mixed infections, especially with Bacteroides species, are common. Penicillin is the drug of choice against the peptostreptococci. In the penicillin-allergic patient, clindamycin or erthromycin is indicated. The anaerobic staphlococcus is generally of low virulence and is uniformly sensitive to penicillin.

Clostridial species are strictly anaerobic, gram-positive rods. The most common species is *C. perfringens* (also known as *Cl. welchii*). Puerperal infection with clostridia is uncommon and may take one of the following forms: (1) a mild endometritis which subsides promptly, (2) myometritis and peritonitis, or (3) septicemia, circulatory collapse, hemolysis, and renal failure. In both postabortal and postpartum infections,[67, 68] it has been emphasized that the simple isolation of this organism from the pelvis does not signify life-threatening infection or the need for hysterectomy. Instead, the initial treatment should consist of antibiotics (penicillin) and supportive therapy. If signs of infection beyond the endometrium do occur, hysterectomy and usually salpingo-oophorectomy are indicated. These signs include worsening of clinical picture or failure to respond promptly, parametrial tenderness, crepitance, myalgia, or hypotension, falling CVP, or oliguria despite adequate volume replacement.

In a review of 70 cases of severe near-term clostridial infections between 1928 and 1959, Ragan[69] concluded that prolonged, obstructed labor appeared to be a predisposing factor. Brown and associates[68] however, reported two cases of sepsis in patients undergoing cesarean section after brief labors.

Characteristically, severe puerperal clostridial infections are marked by rapid onset of fever, abdominal pain, uterine tenderness, a foul-sweetish odor in the lochia, typical forms on the gram stain, and usually high white blood cell counts. Presence of gas in the uterus on x-ray occurs late, if at all.[68] Since many other organisms produce gas, this x-ray sign may not be interpreted as pathognomonic of clostridia. Despite vigorous therapy, including hysterectomy, the mortality rate in these infections is high.[68–71]

Anaerobic gram-negative rods: Bacteroides. Recent reports have paid much attention to these strictly anaerobic, gram-negative bacilli.[72, 74] These infections tend to occur in traumatized and devitalized tissue, producing a foul odor, and tend to produce protacted illnesses, often complicated by septic pelvic thrombophlebitis and occasionally septic embolization.[73, 75]

Life-threatening infection with Bacteroides species occurs infrequently. Ledger and associates[73] reported two cases in over 20,000 deliveries, and Bosio and Taylor[74] reported three cases in over 11,000 deliveries and three others transferred to their institution. Four of these eight infections followed cesarean section. All patients had multiple surgical procedures and long hospitalizations, but none died. Fellner and Dowell[75] reported a 32 per cent mortality rate for Bacteroides bacteremia in general, but many of their patients had some underlying debilitation. In obstetric patients, the outcome would seem to be much more favorable.

The commonest species is *B. fragilis*, which is usually sensitive to clindamycin and chloramphenicol and, to a lesser degree, tetracycline.[75] Other species are usually sensitive to penicillin.

Other anaerobic gram-negative bacilli such as Fusobacteria have been cultured less frequently. These are sensitive to penicillin, erthromycin, or tetracycline.

Mycoplasma hominis and T-form mycoplasmas. Mycoplasma are microorganisms distinctly different from bacteria in that they have no cell wall. Mycoplasmas also differ from bacterial L forms, which are also cell-wall deficient. The colonial morphology of mycoplasma is the typical "fried egg" configuration, measuring about 100 μ in diameter (barely visible with the unaided eye). T-form mycoplasmas (T for "tiny") are so called because their colonies are only 20 to 30 μ in diameter.

Of the eight recognized types, two (*M. hominis* and T mycoplasmas) are involved in genital tract infection. *M. hominis* is sensitive to tetracycline and lincomycin, but resistant to erthromycin. T mycoplasmas are sensitive to tetracycline and erthromycin, but resistant to lincomycin. The role of these organisms in genital infections has recently been reviewed by McCormack and colleagues.[76]

T forms and *M. hominis* are found in the cervix of a large percent of women registering at prenatal clinics.[77] It appears that both *M. hominis* and T forms are capable of causing amnionitis, endometritis, and wound infections.

The evidence for this has been both indirect and direct. In prospective studies, a higher incidence of postpartum fever has been noted in patients who had

M. hominis cultured in the vagina antenatally.[78, 79] Conversely, it has also been shown that patients with postpartum fever are more likely to have *M. hominis* recovered from their cervix and/or a significant rise in antibody titer.[80]

More direct evidence has come from reports of isolating *M. hominis* or T forms from blood cultures and the demonstration of rising antibody titers in patients with puerperal fever or amnionitis.[81–85]

Solomon and co-workers[86] have also found T forms and *M. hominis* in pure culture from wound pus after cesarean section. These specimens were not routinely cultured for anaerobes, however, and thus the possibility of mixed infection with anaerobes could not be ruled out.

The clinical picture of mycoplasma septicemia is characterized by high temperature, spiking to 103° F., and few localizing signs, in a patient who appears nontoxic. The lochia is nonfoul unless anaerobes are present also. White blood counts are reported from 5,800 to 13,000. There is no response to multiple antibiotics such as penicillin, ampicillin, cephalosporins, and kanamycin, but there is a prompt response (in about 48 hours) to tetracycline (in the absence of an abscess).

Because cultures for mycoplasma are done in only a few clinical laboratories, it is impossible to estimate the over-all role of these organisms in puerperal infection.

CHARACTERISTICS OF UNCOMMON ORGANISMS. Over the past few years, a number of other organisms have been reported to be associated with puerperal infection. Appearance of some of these organisms has raised concern of a changing spectrum of bacteria in pelvic infection. For the most part, however, the cases appear to be isolated occurrences.

Members of the hemophilus group have caused puerperal bacteremia in a few instances. These aerobic (or facultative), pleomorphic, gram-negative rods have rather fastidious growth requirements and produce very tiny colonies, which may be overlooked at even 48 to 72 hours or obscured in mixed culture.[87]

Hemophilus vaginalis is frequently isolated from the vagina or cervix and generally thought to cause many cases of "nonspecific" vaginitis. In 1959, Edmunds[88] demonstrated that patients with postpartum temperature elevations were more likely to have this organism in their vagina than those without a temperature elevation. Patients with temperatures over 100° F. had the highest incidence of *H. vaginalis*. Since nearly all the temperature elevations were of a low degree, however, it is difficult to be certain that any of these patients were infected. More convincing evidence for the occasional systemic pathogencity of this organism was its isolation in blood cultures from two patients who had puerperal infection after cesarean section. Both patients responded quickly to ampicillin, the drug of choice.[87, 89]

Additional cases of obstetric septicemia with *H. vaginalis* (alone or in combination with anaerobic organisms) were reported recently.[90]

Another species, *H. influenzae*, is a well-known agent in childhood and adult infections including meningitis, endocarditis, pneumonia, and pyelonephritis. It has been found in vaginas of children, but not adults. Ellner and Shahidi[91] noted two cases of puerperal bacteremia with *H. influenzae* over 5 years. One bacteremia was pure; the other was mixed with anaerobic streptococci and Bacteroides.

H. parainfluenzae, a normal inhabitant of the upper respiratory tract, has also been recovered from the vagina and duodenal secretions and may cause endocarditis. In 1972, Zinner and co-workers[92] reported a case of *H. parainfluenzae* bacteremia occurring immediately postpartum. The mother developed a fever to 102° F. a few hours later. *H. parainfluenzae* was not found in the lochia; the blood culture was not repeated, and her titers to *H. parainfluenzae* were of questionable significance. The neonate developed sepsis and showed a significant rise in titers, however.

Listeria monocytogenes, an aerobic gram-positive rod, is widely found in both man and animals.[93] In man, it may cause meningitis, especially in debilitated hosts. A number of documented cases of lethal intrauterine infections, with intact membranes, or severe neonatal infections have been reported. Often the mother is completely asymptomatic and merely has the organism cultured from her vagina. She may have minimal signs of infection (low-grade fever and slightly elevated white blood cell count).[94–96] On occasion, *L. monocytogenes* has caused maternal bacteremia in association with antepartum fevers[97] and in association with severe maternal postpartum infection with septic shock.[93] A role has also been suggested for Listeria in repeated abortions and prematurity,[95] but Hood[97] was able to isolate it only once in hundreds of cultures taken from the cervix at prenatal visits, normal placentas, meconium of premature infants, or the pelvis in cases of spontaneous abortion or postpartum fever.

In 1972, Ledger and Headington[98] reported the first isolation of *Pseudomonas pseudoalcaligenes* in a severe pelvic infection in which the primary pathogen was clostridia. Previously, this species had been found in blood, pus, urine, feces and the human respiratory tract.

Although there is much concern about development

of superinfection in patients on prolonged antibiotic therapy, this appears to be a rare occurrence in obstetric patients. Masterson and colleagues[99] described a case of lethal monilial septicemia and pneumonia in a patient who was one month post partum; however, the patient was a compromised host with progressive renal failure from malignant nephrosclerosis.

Tetanus after term delivery must be extremely rare in developed countries, but recent cases from South Viet Nam and Nigeria have been reported.[100, 101]

Two reports of viral illnesses involving postpartum patients seemed coincidental to the pregnancy. German and colleagues[102] described an outbreak of febrile illness with rash among maternity patients and neonates in a Royal Air Force Hospital in Singapore. Viral isolates and antibody titers suggested ECHO virus as the agent. In 1970, O'Neill and Schwarz[103] reported a case of cytomegalovirus causing delayed posttransfusion fever in a patient with a large puerperal retroperitoneal hemorrhage.

Urinary tract infection

Some reports have found urinary tract infection to be the most common source of postpartum fever[8, 12] whereas others have found it to be second in frequency after genital tract infection.[3, 9]

As there are difficulties in diagnosing genital tract infection, so too there are problems in diagnosing urinary tract infection. The first problem is defining urinary infection. Since diagnostic criteria are frequently not mentioned, it is difficult to determine whether an author is referring to simply bacteruria, to lower tract infection (cystitis), or to upper tract infection (pyelonephritis.) Moreover, recent studies employing bacteruria-localizing techniques have shown a poor correlation between traditional clinical diagnostic criteria and source of bacteruria.[104] Indeed, up to 40 per cent of ambulatory patients with acute frequency and dysuria may have a sterile culture.[105] No similar information is available for puerperal women.

Despite these limitations, it is apparent that postpartum females are predisposed to symptomatic urinary infection because of catheterization in labor[106] and because of antecedent asymptomatic bacteruria (2 to 7 per cent of pregnant women).[107] Though it is beyond the intent of this review, thorough discussions of asymptomatic bacteruria in pregnancy are available.[107]

A presumptive diagnosis of urinary infection may be based on traditional signs and symptoms (frequency, dysuria, back pain, fevers, chills, costovertebral angle tenderness, and pyuria). To avoid contamination of voided specimens, supervision of the patient is important.

Since antibiotic therapy is often started prior to receiving the culture report, a knowledge of the likely etiologic organisms is essential. In the vast majority of cases, *E. coli* is the pathogen. In other cases, the organism may be Proteus, Klebsiella-Enterobacter, or enterococci. On occasion, Pseudomonas, staphlylococci (both coagulose positive and negative), and streptococci (other than enterococci) may be pathogenic, while diphtheroids, lactobacilli, and micrococci are contaminants. Anaerobic bacteria rarely cause urinary infection. Antibiotic sensitivity patterns of the bacteria have been discussed previously. While many of these organisms are sensitive to sulfonamides, which reach high levels only in the urine, the initial antibiotic in febrile patients should be one with high blood levels as well since bacteremia is possible. Parenterally administered ampicillin or a cephalosporin may be used initially, with an aminoglycoside if there is septic shock or a history of recurrent infection. Antibiotic use may then be modified on the basis of culture and sensitivity results and clinical course.

Nearly all patients respond clinically within 48 hours, and bacteruria should be cleared within 72 hours of appropriate therapy. Failure to respond suggests obstruction or interference with treatment by an indwelling catheter. Also, adverse urinary pH may inactivate some antibiotics. Recommended treatment of primary infections is for 10 to 14 days.

Follow-up urine cultures should be obtained 1 to 2 weeks after cessation of therapy and again in a few months. If bacteruria persists or recurs, further evaluation is indicated—including an intravenous pyelogram, cytoscopy, and bacteruria-localizing studies.

Other sources of postpartum infection

There are few peculiarities to puerperal abdominal wound and pulmonary infections, and these will not be discussed here.

Occurring relatively late in the puerperium, breast infections are manifested by typical signs and symptoms of cellulitis or abscess and usually present little difficulty in diagnosis. Because the most common pathogenic organism is a coagulase-positive staphylococcus (either penicillinase or non-penicillinase producing), the initial drug of choice is a penicillinase-resistant penicillin derivative. If the culture of the milk shows that the organism is sensitive to penicillin, then this agent should be substituted. In the penicillin-allergic patient, cephalosporins may be used. Erythromycin is another alternative, but staphylococci may rapidly develop resistance to it.

Infections resulting from spinal or epidural anesthesia must be very infrequent as none was found in over 10,000 spinal blocks[108] or over 32,000 epidural blocks.[109] Male and Martin[109] reported a case of spontaneous epidural abscess in a patient who did not have conduction anesthesia.

Within the last few years, a number of cases of subgluteal or retropsoal infections have been reported.[110, 111] These severe infections had one common feature—the use of paracervical or transvaginal pudendal block. They were characterized by poorly localized hip or pelvic pain of variable onset and fever. Gas formation in the soft tissues was seen in half the cases. Many patients had extensive hospitalizations (up to 80 days), and two of nine died. Since six of seven confirmed cases occurred on the left, it has been suggested that rectosigmoid penetration leads to a nidus of infection, which then spreads laterally or superiorly. Responsible organisms were gram-negative aerobes (with *E. coli* most common), anaerobic streptococci, and Bacteroides. Besides appropriate antibiotic treatment, early debridement, possibly with diverting colostomy (with colonic-peritoneal fistulas) will be necessary.

Eder[112] described a case of protracted postpartum sepsis caused by gram-negative osteomyelitis of the right iliac crest. The hospitalization lasted 88 days. No etiology was clear, but the patient had noted night sweats and fevers during the last 2 months of pregnancy.

Acute hematogenous tuberculosis has recently been reported as an unusual cause of immediate puerperal fever in New York City.[113] The patient's course was marked by high spiking fevers, low white blood counts, negative cultures, and failure to respond to the usual antibiotics. Her chest x-ray was normal until 5 weeks after the onset of fever. *M. tuberculosis* was cultured from maternal sputum and urine.

Some infections appear to be more likely to occur during pregnancy or the puerperium. In Nigeria, pregnancy is known to predispose to amoebic infections.[114] In 1972, a case of fatal amoebic colitis and peritonitis was reported in a young woman who had spent her entire life in the Washington, D. C., area.[115] In underdeveloped areas, puerperal and pregnant women also appear more subject to pneumococcal and tuberculous meningitis.[116, 117]

Special complications of puerperal infections

From the above descriptions, it is clear that on occasion the obstetric patient is stricken with life-threatening infection and is subject to septic shock, renal failure, and disseminated intravascular coagulopathy.[118–120] While a thorough discussion of these complications is beyond the scope of this review, a few features of septic shock deserve emphasis. First, the most likely causative organism is *E. coli*, but other gram-negative aerobes, clostridia and Bacteroides, are possible. Appropriate antibiotic coverage must therefore be directed at all likely pelvic organisms. Second, although the reported incidence of death from septic shock is about 65 per cent in large series,[118] many of these patients have an underlying debilitating disease. In obstetric patients, the over-all mortality rate would appear to be much lower, perhaps only 20 to 25 per cent. Finally, one must consider the need for surgical treatment. In septic shock following term delivery, the primary mode of therapy is supportive—fluids, antibiotics, vasoactive drugs, steroids, and close monitoring. If this treatment fails to bring about a response within 4 to 12 hours,[119, 121] then surgery is indicated. In the moribund patient, this interval must be even shorter. Unlike the shock accompanying septic abortion, septic shock with term delivery usually involves infection extending beyond the uterine cavity. Therefore, curettage may be performed first, but unless large amounts of necrotic placental tissue are unexpectedly recovered, an abdominal hysterectomy and often bilateral salpingo-oophorectomy with drainage will be the procedure of choice.

One other complication, septic pelvic thrombophlebitis, has been widely recognized recently. This entity was first recognized over 35 years ago. In 35 per cent of autopsies of patients dying of puerperal sepsis, pelvic thrombophlebitis was discovered, and 50 per cent of the deaths attributed to puerperal sepsis were due to pulmonary emboli.[122] In 1950, Collins and co-workers[122] reported on 70 patients with suppurative pelvic thrombophlebitis treated with ligation of the inferior vena cava and ovarian vessels. Suppurative pelvic thrombophlebitis, it was suggested, led to repeated small septic emboli and septicemia rather than sudden massive pulmonary emboli and death. At laparotomy, clots were found equally distributed among the ovarian, cervical, and hypogastric vessels and equally between the right and left sides. Two thirds of the patients had chills and fever, especially a "picket-fence" pattern, and many had tachycardia and tachypnea. Chest pain, cough, or hemoptysis was seen in over one third. The pelvic examination was usually normal, with a tender thrombosed vein being palpable occasionally. The side of the phlebitis could be predicted in only 60 per cent, however.

Cases of septic pelvic thrombophlebitis may be divided into two distinct syndromes with different onsets, symptoms, and findings on examination.

One is a syndrome[123–134] characterized by early onset (usually within 2 to 3, but up to 5 days following delivery), severe pain, a normal to high temperature, and findings of an acute abdomen. In about half the cases, there is a palpable mass. In all cases, the abdominal findings were so impressive that a laparotomy was performed with a preoperative diagnosis usually of acute appendicitis, twisted ovarian cyst, or pelvic abscess. In few of these cases is the diagnosis of pelvic thrombophlebitis considered.[125, 131] When the diagnosis is recognized at laparotomy, a variety of procedures have been performed, from ligation of the ovarian veins and vena cava to unilateral salpingo-oophorectomy. In one case, the abdomen was closed immediately, and the patient was treated with heparin.[127] In nearly all cases, the phlebitis is on the right side. Postoperative treatment also varies. Some patients are given heparin or antibiotics alone or together; others receive no special treatment. Probably the best treatment, when the diagnosis of ovarian vein thrombophlebitis is made only in the operating room, is ligation of the ovarian vessels bilaterally and institution of both heparin and antibiotics.[136, 137]

The second symdrome[135, 136, 138, 139] is much less dramatic and appears as an "obscure" or "enigmatic" fever persisting despite multiple antibiotics. These patients characteristically appear ambulatory and nontoxic with either no pain or mild, poorly localized discomfort despite wide swings in temperature. Abdominal and pelvic findings are minimal and vague. In all cases, however, the diagnosis has been presumptive and has been based on rapid defervescence (within 48 to 72 hours) after initiating heparin therapy. In some cases, the response to heparin is clouded by concomitant change in antibiotics.

Sweet and Ledger[8] recently noted that 8.6 per cent of their patients with postpartum fevers were treated with heparin for a presumed diagnosis of pelvic thrombophlebitis. Fellner and Dowell[75] reported that 30 per cent of patients with Bacteroides bacteremia experienced embolization.

Blood cultures, in either syndrome, may be helpful. In four reports reporting blood culture results, 16 of 58 (27 per cent) were positive.[122, 127, 135, 138] Organisms found were aerobic and anaerobic streptococci, *S. aureus*, *E. coli*, and Bacteroides. In either type of syndrome, the duration of anticoagulant therapy (either heparin alone or followed by coumadin) has been a few days to 3 weeks.

Developments in therapy

Prophylaxis. The debate on the use of "prophylactic" antibiotics in obstetrics began in the late 1940's. In a series of articles, Keetel and co-workers[140–142] reported that prophylactic administration of penicillin to all patients in labor resulted in a decrease in the incidence of significant fevers from 6.3 to 2.4 per cent. As noted earlier, it was implied in a double-blind study with PROM that prophylactic tetracycline could lower the puerperal infection rate in all gravidas.[30] Despite these studies, the general use of prophylactic antibiotics never became accepted for a variety of reasons. First, the general incidence of infection is low, and infections are rarely severe. Thus, up to 95 or 97 per cent of patients would receive antibiotics needlessly so that perhaps 2 to 4 per cent would be spared infection and another 2 per cent would still become infected. Second, there are recognized dangers of broad use of antibiotics such as serious or even lethal drug reactions and emergence of resistant organisms. Indeed, Hill[4] reported that on an obstetric service when prophylactic antibiotics were discontinued there was a fall in the incidence of antibiotic-resistant staphylococci and a fall in the incidence of staphylococcal infection. As in many areas of medicine, it has been the general feeling in obstetrics that administering prophylactic antibiotics routinely is uneconomical, unnecessary, and unwise.

In certain groups at risk for infection, prophylactic antibiotic administration has been more widely accepted. Because of the development of endocarditis in patients with damaged heart valves or cardiac prostheses, it is standard practice to give broad-spectrum antibiotics while in labor and for a few days postpartum. The incidence of asymptomatic bacteremia is less than 2 per cent when serial samples have been drawn at intervals from immediately postpartum to 72 hours postpartum.[143–145] This must be considered to be a minimum, however, in view of the short sampling periods.

Other patients considered for prophylaxis are those with prolonged ROM and most studies concerning this group of patients yield conflicting results. Burchell[26] found that administering prophylactic antibiotics (type not specified) seemed to decrease PNM and maternal fever, especially when the latent phase was over 48 hours. Here, the group without antibiotics had a 21 per cent morbidity rate compared to a 10 per cent morbidity rate in the antibiotic group. Thus the vast majority (nearly 80 per cent) even in the control group had no fever. In a double-blind study,[30] the use of prophylactic tetracycline decreased maternal morbidity in patients with PROM. For patients receiving placebo, the over-all morbidity was 7.3 per cent; for those receiving tetracycline, it was 3.5 per cent. Again, the overwhelming majority of patients even without antibiotics (96.7 per cent) still had no morbidity. Maternal

morbidity was not correlated at all with the latent phase of labor. Of 515 patients with PROM in premature pregnancies reported by Breese,[25] 142 received antibiotics (penicillin, streptomycin) but at the discretion of the staff. Although there appeared to be an improved PNM in those receiving antibiotics, the evidence was unclear.

Other studies have shown no value of prophylactic antibiotics. Humphrey[10] found that the use of antibiotics (type not specified) when labor or ROM was greater than 18 hours made no statistically significant difference in maternal infection rate. Of 122 patients without antibiotics, 15.5 per cent became infected whereas 12 per cent of only 33 with antibiotics became infected ($P > 10$ per cent). In another report, nitrofurozene vaginal suppositories were also found to have no effect when used with ROM of more than 6 hours.[146]

Since other studies have reported maternal morbidity as high as 30 per cent with prolonged ROM even with prompt induction, a prospective investigation of prophylaxis under these conditions would be of value.

Within the past 6 years, the use of prophylactic antibiotics in cesarean section has generated much interest. In 1968, Miller and Crichton[147] employed ampicillin pre- and postoperatively. In 300 cases, they found a decrease in over-all febrile morbidity from 64 to 26 per cent. Patients in the placebo group were started on antibiotics if a temperature of only 99° F. or over persisted, a practice potentially leading to the overdiagnosis or morbidity and overuse of antibiotics in the control group.

In a second report, Weissberg and colleagues[148] administered penicillin and kanamycin to patients undergoing cesarean section while in labor. A decrease in the total number of complications and a shortening of hospital stay was noted. The study was not blinded, however, and treatment was not standardized. Next, Morrison and colleagues[149] found a decrease in morbidity from 51 per cent in control subjects to 22 per cent in treated patients when penicillin and kanamycin were used for 3 days on 230 patients. A decrease in "severe infections" was also noted. Finally, in two reports, Gibbs and co-workers[150–151] found in a double-blind study of 129 patients that the use of an 8 hour perioperative course of ampicillin and kanamycin decreased febrile morbidity from 63 to 25 per cent ($P < 0.002$). These results compared favorably to those of the previous studies in which the course of antibiotics was 3 to 5 days.

In a review of prophylactic antibiotics in cesarean section, a much lower morbidity rate was noted in Great Britain.[152] Even when cesarean section was performed after 24 hours of ROM, the morbidity rate was only 5 to 12 per cent. This discrepancy may be based on different socioeconomic status of the population studied, since the American studies involved indigent patients. It was acknowledged that the patient would have more discomfort if infected, but most infections were "curable and can be nipped in the bud with clinical vigilance and early treatment. However, they can have unfortunate sequelae in infertility, kidney damage, wound dehiscence, lung damage, and possibly subsequent uterine rupture and ventral hernia. . ." Finally, the potential of producing resistant organisms was again raised.

Since many prophylactic regimens are initiated prior to delivery, antibiotics commonly may be expected to achieve therapeutic levels in the fetus. There have been no reports of adverse fetal effects, but it remains for this point to be systematically evaluated.

Despite faults in design of these experiments, it appears reasonable to conclude that in selected groups of cesarean section patients (with baseline morbidity of 50 to 60 per cent) prophylactic antibiotics are of value.

Surgical treatment of infection. As Ledger has noted recently, few guidelines have been established for surgical intervention in treating severe obstetric and postoperative infections.[153] In a discussion of 33 such cases, including eight obstetric cases at the University of Michigan, it was emphasized that the decision for operation should be based upon a persistently febrile course not resolved by appropriate systemic antibiotics and possibly heparin. At laparotomy, all patients had clear evidence of infection. Of the eight postpartum infections, five followed abdominal delivery (one cesarean-hysterectomy and four low vertical sections) all in labor. Others have indicated the need for laparotomy in similar situations.[154, 155] The indications for surgery in septic shock and clostridial infections have been discussed earlier.

Other developments. Recently, the concept of an infection surveillance team to detect changes in prevalent organisms in both sporadic and epidemic infections was described at the University of Michigan.[156] Such a surveillance system uncovered a highly significant association ($P < 0.005$) between conduction anesthesia and urinary tract infections. Another new concept is the fever index,[157] a numerical expression of the total quantity of fever, expressed in degree hours. The index is computed as the area of the fever curve above 99° F. This offers a number of advantages over standard morbidity in identifying morbid groups and also quantitatively evaluates response to therapy.

Treatment of refractory fever. The febrile puerperal patient who fails to respond to the "usual"

antibiotics requires a logical and thorough evaluation.

The initial question in evaluating this problem is: "Is the cause of the fever infection?" Noninfectious etiologies include drug fever, factitital fevers, and rarely concomitant collagen disorder or tumor. A detailed history and thorough examination are essential in answering this question. Culture results should be evaluated as well as the white blood count and white cell changes (increases of immature granulocytes, toxic granulations, and eosinophillia). The nitroblue tetrazolium reduction test has been of questionable assistance.[158–159]

If infection appears likely, the next question is: "Is the source the genital tract or elsewhere?" Sources such as appendicitis, endocarditis, tuberculosis, and urinary tract infection must be ruled out. The third question is: "Have the antibiotics been appropriate, and has the course been long enough and intensive enough?" The answer clearly lies in culturing the *endometrium* (not simply the cervix) and blood properly for anaerobes and perhaps mycoplasma. If inadequate culturing technique has been used in previous genital tract samples, then it is often appropriate to stop antibiotics and reculture in 24 to 48 hours. In refractory fevers, the antibiotics must be given parenterally.

When the antibiotic selection and dosage appear adequate, the next question is: "Is there pelvic thrombophlebitis?" This entity has a characteristic picture. When the diagnosis is in doubt, a short trial of intravenous heparin may be indicated.

All during this re-evaluation, the patient should be examined every 24 to 48 hours for evidence of a localized collection of pus or blood which should be drained. Because tenderness and guarding may obscure a mass, an examination under anesthesia may be considered. If the patient continues to have high spiking fevers, then an exploratory laparotomy may well be indicated.

Summary and conclusion

Despite advances in obstetrics and the availability of potent antibiotics, the incidence of puerperal infection has not decreased during the last 20 years, and infection has remained a leading cause of maternal mortality.

Sources of postpartum infection are primarily the genital and urinary tracts. Because of varying criteria employed, the incidence varies from 1 to 8 per cent. Group A β-hemolytic streptococcal outbreaks still occur, infecting up to one third of patients. Furthermore, selected groups, especially those undergoing cesarean section, those with premature rupture of the membrane, and those of low socioeconomic status have morbidity rates far above the over-all average.

A variety of organisms is found even in the normal pelvis, including the normal postpartum endometrium. Many difficulties are present in establishing the clinical and microbiologic diagnosis of puerperal endometritis. Over the last 20 years, *E.coli* has been recovered with increased frequency, but streptococci still are major pathogens. Other common pathogens are staphylococci and bacteroides and, on occasion, other gram-negative aerobic bacilli, clostridia, or mycoplasma may be the primary pathogen.

There are clinical problems in both defining and localizing urinary tract infections. By far, *E.coli* is the most common pathogen, although other gram-negative aerobes and enterococci may also be pathogenic. Therapeutic recommendations are presented.

Unusual sources of postpartum infection including abscess of the epidural, retrosoal and subgluteal spaces, acute hematogenous tuberculosis, and amoebic colitis have been reported recently. Special complications of severe infection are noted, and special attention is given to septic pelvic thrombophlebitis.

Finally, developments in therapy are reviewed. Major areas of interest are prophylactic antibiotics, especially with prolonged rupture of the membranes and with cesarean section, and surgical intervention in severe infection.

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Certification by the Division of Reproductive Endocrinology, the American Board of Obstetrics and Gynecology, Inc.—November, 1975

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Certification by the Division of Maternal-Fetal Medicine, the American Board of Obstetrics and Gynecology, Inc.—November, 1975

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Current Concepts in Management of Gynecological Cancer

Indiana University School of Medicine, Departments of Obstetrics and Gynecology and Radiotherapy, announces a two-day symposium, "Current Concepts in Management of Gynecological Cancer," to be held in Indianapolis, Indiana, May 20-21, 1976. Guest faculty will include: Philip J. DiSaia, Paul B. Underwood, Louis Delclos, Charles Boyce, Carlos Perez, and Helmut Schellhas. A registration fee of $75 will be charged. For further information, write or call: John Roscoe, Division of Postgraduate Education, 1100 W. Michigan St., Indianapolis, Indiana 46202. Telephone: (317) 264-8353.

Pediatric and Adolescent Gynecology

The Page and William Black Post-Graduate School of Medicine of the Mount Sinai School of Medicine (City University of New York) announces a post-graduate course entitled "Pediatric and Adolescent Gynecology," to be held June 3 and 4, 1976, from 9:00 A.M. to 5:00 P.M. (two sessions) at The Mount Sinai Medical Center, Fifth Ave. and 100th St., New York, New York 10029. The course will be directed by Albert Altchek, M.D., and guest faculty. Fourteen hours of credit in Category 1 toward the American Medical Association Physicians' Recognition Award will be given. The course is acceptable for 15 prescribed hours by the American Academy of Family Physicians and has been accredited for 20 cognates by the American College of Obstetricians and Gynecologists. A reduced fee is available for house staff. Application should be made to: Director, The Page and William Black Post-Graduate School of Medicine, Mount Sinai School of Medicine, Fifth Ave. and 100th St., New York, New York 10029. Telephone: (212) 650-6737.

Sixth Annual Meeting of the Great Plains Organization for Perinatal Health Care

The Sixth Annual Meeting of the Great Plains Organization for Perinatal Health Care will be held May 2-4, 1976, at the Radisson South Hotel, 7800 Normandale Blvd., Minneapolis, Minnesota 55435. The theme will be "Psycho-Social Aspects of Perinatal Crisis." Featured speakers and their topics are: LaVohn Josten, R.N., Minneapolis Health Department, "Parenting"; Joseph Fletcher, S.T.T.D., Department of Medical Ethics, Baylor University, and Thomas Sullivan, Ph.D., National Endowment for the Humanties at Brown University, "Moral and Ethical Dilemmas"; Dr. Haper, Marshfield Clinic, Department of Psychology, "Parent's View of Perinatal Crisis"; Stanley Graven, M.D., Professor, University of Wisconsin, "Five Years of Perinatal Medicine—Is It Really Worth It?" Sixteen workshops will be held: "Diets and Fads in Pregnancy," Roy Pitkin, University of Iowa; "LeBoyer Method of Delivery"; "Surgical Emergencies

in the Neonate"; "Group B Streptococcus Sepsis"; and "Induction of Pulmonary Surfactant." "Problem-Solving Booths" will be manned for questions, in-depth discussion, and interchange among disciplines regarding perinatal care and regionalization. Displays of available equipment will be held on both days. The fees are: $16 for members of the Great Plains Organization (includes two lunches) and $25 for nonmembers (includes two lunches). For further information, contact: Mrs. Linda McNally, Great Plains Organization, 202 S. Park St., Madison, Wisconsin 53715. Telephone: (608) 267-6990.

Canadian Andrology (Male Reproduction) Symposium

The first Canadian Symposium on "Biology of Male Reproduction" will be held on June 7, 1976, at Mount Sinai Hospital, Toronto, Ontario, Canada. The program, organized by E. S. E. Hafez (Detroit). B. N. Barwin (Ottawa), and J. Bain (Toronto) will deal with diagnosis of male infertility; anatomic, physiologic, endocrine, biochemical, immunologic, pathologic, and psychiatric mechanisms of male infertility; and male sexuality. Deadline for registration is May 15. For further information, please contact: Dr. J. Bain, Mount Sinai Hospital, 600 University Ave., Toronto, Ontario M5G 1X5, Canada. The Canadian Andrology Society will be initiated in conjuction with the symposium.

Safe Alternatives in Childbirth

"Safe Alternatives in Childbirth," a national conference, open to the public, will be held Saturday, May 15, 1976, in Washington, D. C. It is sponsored by NAPSAC, the National Association of Parents & Professionals for Safe Alternatives in Childbirth. Conference topics will include: Maternity practices in other countries; Alternatives in childbirth and infant outcome—a pediatric view; Statistics on home births in the United States; Maternity and childbearing centers—an alternative to both hospitals and home births; Family-centered maternity care in hospitals; The lay midwife; Legal aspects of home births; A working home-birth program—details of the arrangements between physicians, midwives, and hospitals; and other topics. For informational brochure and registration form, contact: Dr. David M. Stewart, Conference Chairman, NAPSAC National Headquarters, Rt. 3, Box 23A, Hillsborough, North Carolina 27278. Telephone: (919) 732-7302.

IVth Symposium of the Fondation pour la Recherche en Endocrinologie Sexuelle et L'Etude de la Reproduction Humaine (F.R.E.S.E.R.H.)

This Symposium will be held in Brussels, Belgium, May 20, 21, and 22, 1976. It is sponsored by the Faculté de Médecine, rue Evers nr 2, 1000 Brussels, Belgium. On Wednesday, May 19, from 7 P.M. to 9 P.M., there will be reception of the participants. On Thursday, May 20, from 8 A.M. to 9 A.M., registration formalities will be conducted. Working sessions will be held from 9.30 A.M. to 12.30 A.M. and from 2.30 P.M. to 5.30 P.M. on May 20, 21, and 22. Topics include: "Steroid hormones during the cycle and pregnancy," "Cerebral monoamines and hypothalamic releasing hormones," "Glucagon during pregnancy," and "Does inhibine exist?" Confirmed participants include: M. Bygdeman, L. Cedard, R. De Hertogh, H. de Watteville, E. Diczfalusy, K. B. Eik-Nes, P. Franchimont, C. Hermier, M. Jutisz, R. Kram, P. Lefebvre, B. Lunenfeld, L. Martini, R. Scholler, and A. Soulairac. Probable participants are: S. G. Johnsen, Sultan M. M. Karim, and Rousseau. Registration fees (payable to account nr 090/9090900/13 of Crédit Communal de Belgique, ref. S/76) are: general, 1.500 F.B.; doctors with less than five years certification, 500 F.B.; students, free. Registration should be directed to: Secrétariat de la Clinique Obstétricale et Gynécologique de l' Hôpital Universitaire Brugmann (Pr. R. Vokaer). Place A. Van Gehuchten, 4-1020 Brussels, Belgium. Entry forms will be forwarded on request: For travel and hotel reservations, please contact: Voyages Montaine (Mr. Verhulst), 32, rue de Tournai, 7700 Mouscron, Belgium. Telephone: 056/333327 or 056/334736. Telex: 57527.

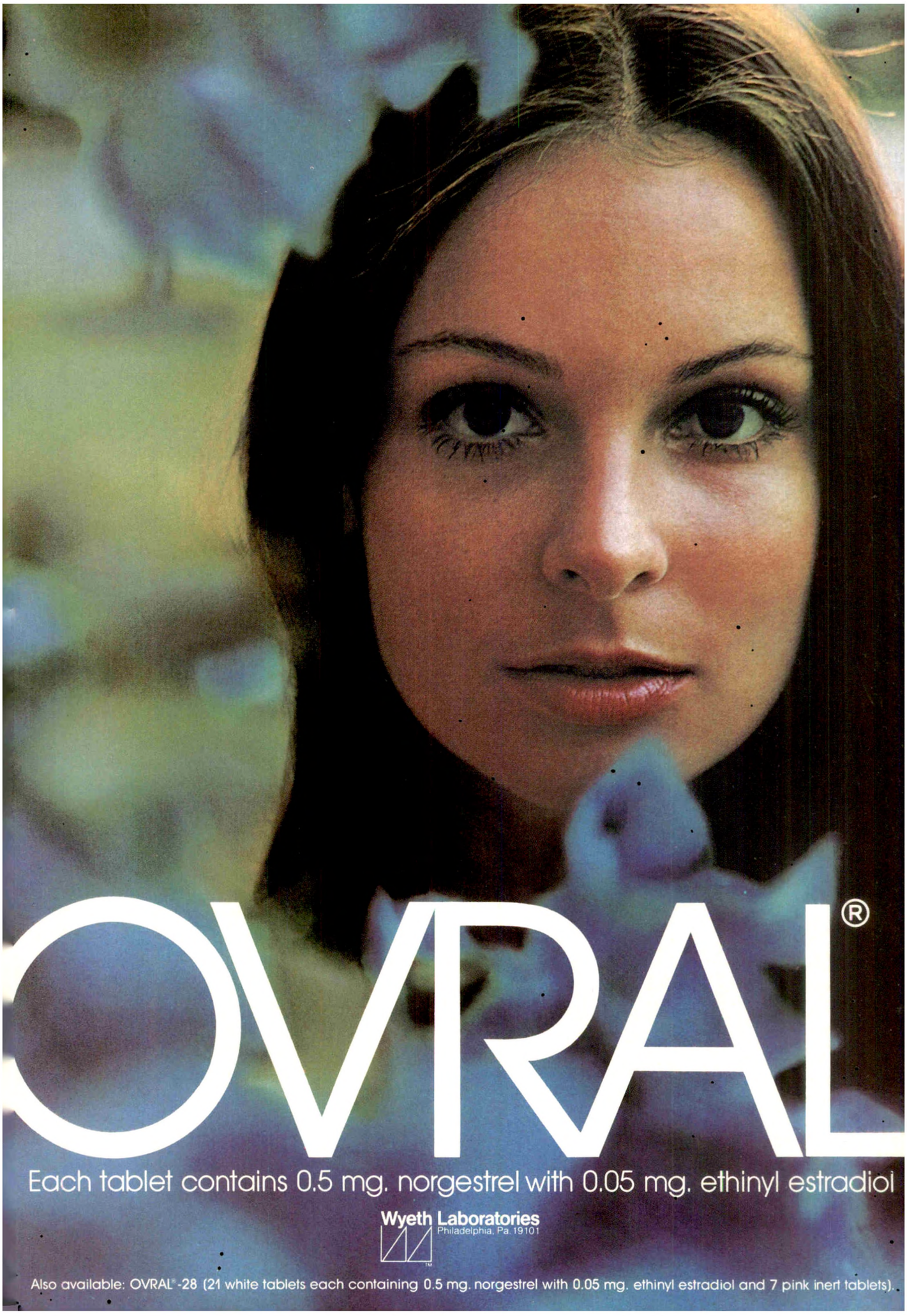
1976 Conference on Clinical Delineation of Birth Defects

The University of British Columbia, Faculty of Medicine, and the National Foundation–March of Dimes will sponsor the 1976 Conference on Clinical Delineation of Birth Defects, June 23-25, 1976. For further information, write to: Dr. R. Brian Lowry, Clinical Genetics Unit, 855 W. 10th Ave., Vancouver, British Columbia V5Z 1L7, Canada.

Advanced course in colposcopy with video tape tutorial, July 22-24, 1976

An advanced course in colposcopy will be held during Montreal's Summer Olympics on July 22-24, 1976, at the Jewish General Hospital, Montreal, Quebec, Canada. Invited speakers include Drs. Duane Townsend, Arthur Herbst, Adolph Stafl, and Ralph Richart.

The registration fee is $250 and includes advanced colposcopy, a session with a live patient, video tape tutorial, and lunches. Registration should be accompanied by a check payable to: Alex Ferenczy, M.D., Registrar, Jewish General Hospital, Department of Pathology, 3755 Cote St. Catherine Rd., Montreal H3T 1E2, Quebec, Canada.


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Microbiologic aspects of fertility...

The greatest potential for bacterial infection in the female genital tract exists during the fertile years. A large proportion of the potentially pathogenic microflora are anaerobic—particularly bacteroides and anaerobic streptococci. Isolation and identification present special problems.

"Pseudo-maturity"

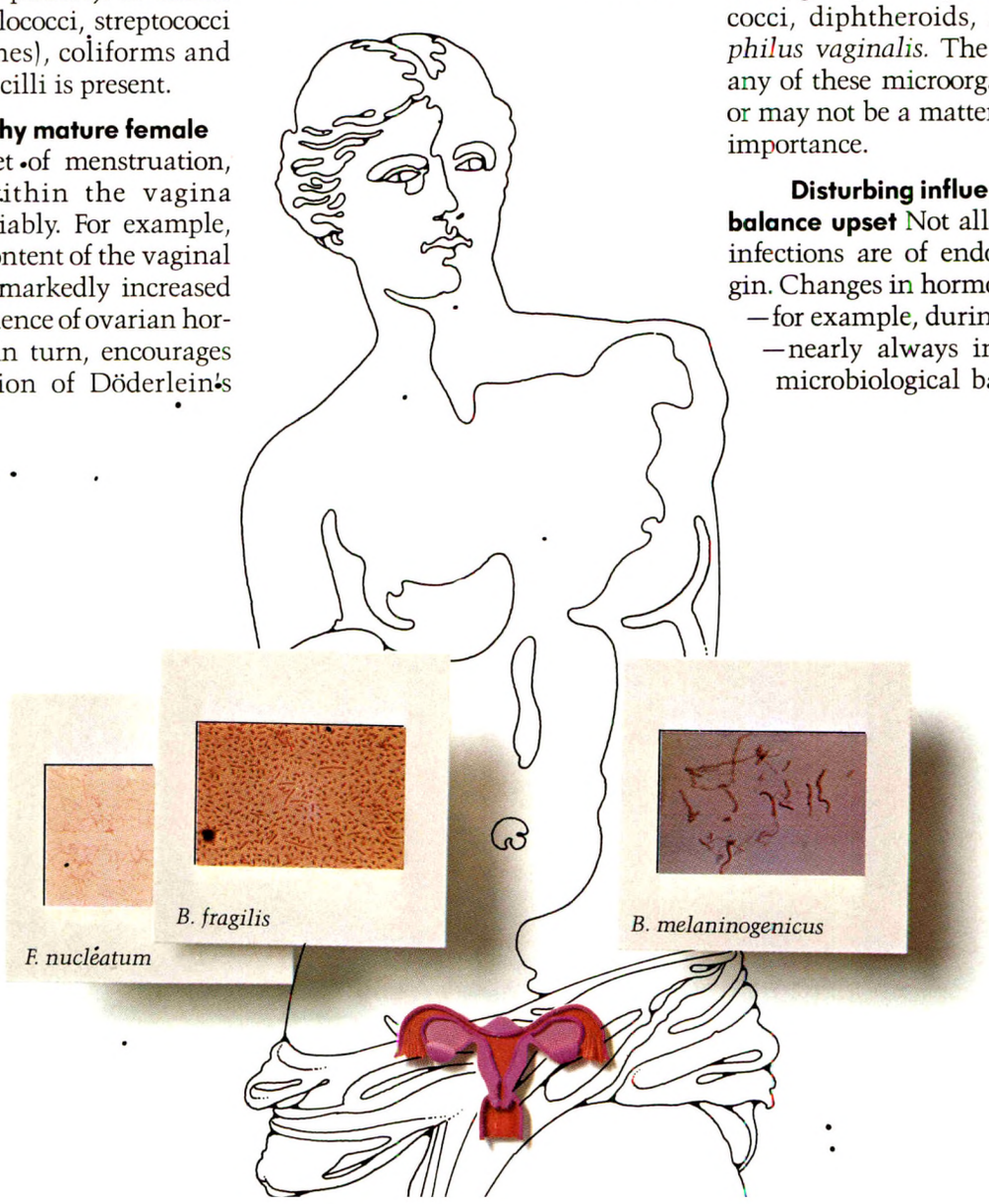
Sterile at the time of birth, the female genitalia begin to develop an abundant bacterial flora within hours. But this maternally influenced flora, composed largely of the familiar gram-positive lactobacillus known as Döderlein's bacillus, disappears in just a few weeks. The vaginal secretions revert toward alkalinity and remain alkaline until puberty. A varied flora of staphylococci, streptococci (except pyogenes), coliforms and diphtheroid bacilli is present.

The healthy mature female

With the onset of menstruation, conditions within the vagina change appreciably. For example, the glycogen content of the vaginal epithelium is markedly increased under the influence of ovarian hormones. This, in turn, encourages the proliferation of Döderlein's bacilli. These lactobacilli create an acid environment which limits the growth of potential pathogens, and the role of lactobacilli in keeping the microflora in balance is vital throughout a woman's reproductive years.

The normal flora—anaerobes predominate Certain other bacteria are also seen regularly in the vagina. With today's improved methods of culturing, it has been demonstrated that a large proportion of these bacteria are anaerobic—notably bacteroides and anaerobic streptococci. Other microorganisms that may be observed are the *Escherichia coli/Enterobacter aerogenes* group of coliforms, the anaerobic micrococci, group B streptococci, enterococci, diphtheroids, and *Hemophilus vaginalis.* The presence of any of these microorganisms may or may not be a matter of etiologic importance.

Disturbing influences—balance upset Not all gynecologic infections are of endogenous origin. Changes in hormonal balance—for example, during pregnancy—nearly always influence the microbiological balance. Both



the resident bacterial population and potential invading pathogens may be affected. Either may be responsible for the occurrence of postpartum infections, postabortal infections, posthysterectomy infections, and the infections that occur concurrently with neoplastic disease. As pathogens ascend the reproductive tract from vagina to uterus to tubes, the risk to the patient is usually enhanced.

Forms of infection

Gynecologists and surgeons alike are well acquainted with such infections: abscesses (whether tuboovarian, pelvic, vaginal, vulval, or Bartholin's gland infection), puerperal sepsis, salpingitis, endometritis, peritonitis of gynecologic origin, vaginal-cuff infection, chronic pelvic inflammatory disease—with each, the threat of possible septicemia. The need for immediate surgical intervention may be controversial, but certain of these infections often represent true gynecologic emergencies.

The problems anaerobes pose...culturing

Improved culturing techniques have demonstrated that anaerobes—especially bacteroides and anaerobic streptococci—inhabit the vagina, as well as more remote genital areas. It has been reported that these usually foul-odor-producing anaerobic organisms may be isolated in three out of four cases of infections of the genital tract. In one series of patients, anaerobes alone were cultured in 37 percent of the cases. Many studies have shown that bacteroides are by far the most common of these organisms, but anaerobes such as peptococci, peptostreptococci and other anaerobic streptococci are also frequently observed. Mixed infections with multiple anaerobic species are not unusual.

Today many physicians suspect that the techniques of obtaining or incubating the culture may be at fault if no anaerobes at all are observed.

A new appreciation of pathogenicity

Only recently has it been realized that anaerobes other than clostridia are pathogens of major significance in serious infections of the female genital tract. Exacting methods of culturing have helped to establish this. One investigation has shown that 44 percent of all anaerobic isolates obtained from a series of 400 patients of all types, not just gynecologic, were associated with infection. Bacteroides and anaerobic streptococci were the most prevalent, with clostridia occurring less frequently.

Whenever these organisms are seen on repeated occasions by Gram stain or isolated by culture—whether alone or combined with other bacteria—they should not be ignored.

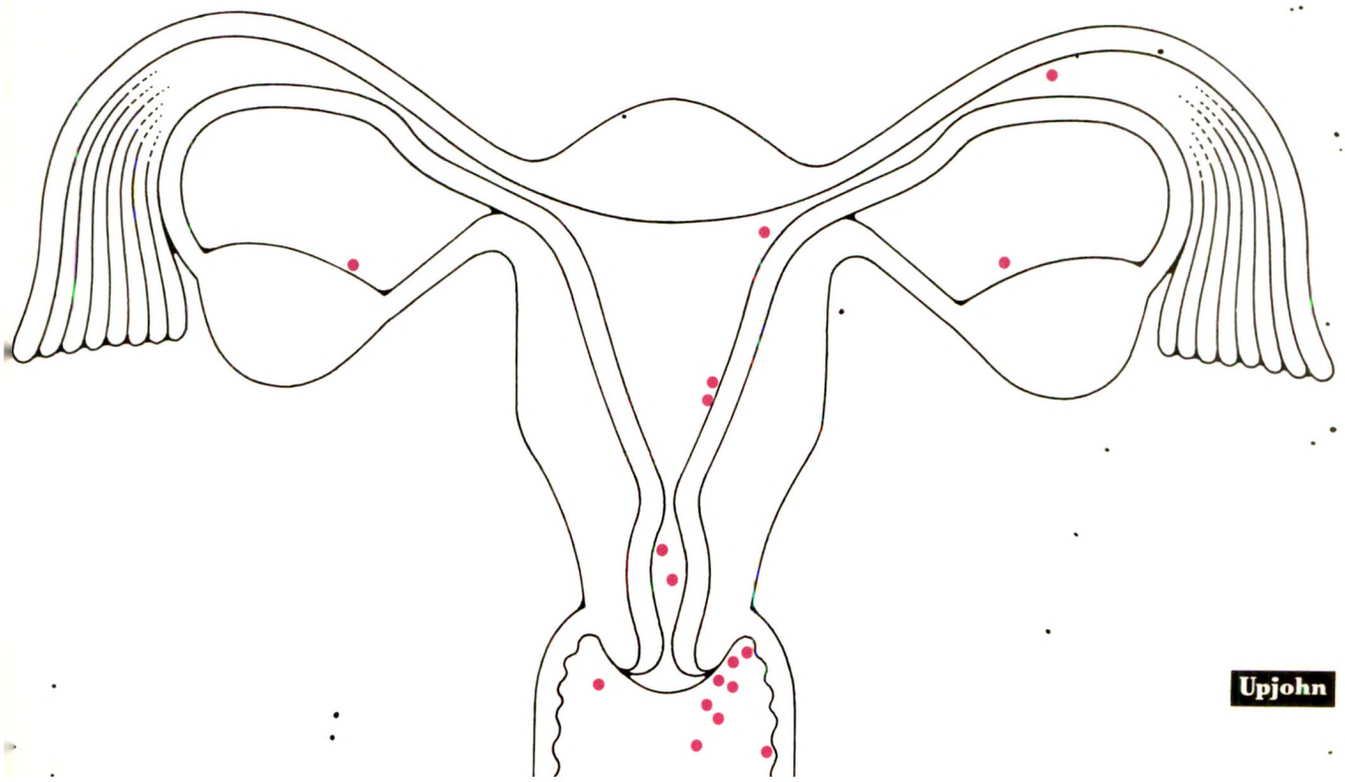
Therapeutic measures

Selection of the most appropriate antibiotic—or antibiotics—is still a problem for the gynecologist in many instances, since many of the infections he deals with cannot be etiologically defined when first observed. Nevertheless, the seriousness of his patient's condition may demand immediate attention. Because there are numerous antibiotics effective against just certain potential pathogens, the physician's problem has been intensified. And for one aspect of his problem—anaerobic infections—there has been no easy answer.

Many gynecologists believe that, of all infections, these present one of today's most serious challenges.

For information on ONE ANTIBIOTIC OF SIGNIFICANCE in the treatment of serious anaerobic infections of the female reproductive tract, please turn page.

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Therapeutic considerations...

Cleocin® Phosphate (clindamycin phosphate injection) has proved to be effective against susceptible anaerobic organisms (whether they are anaerobic cocci or anaerobic gram-positive or gram-negative rods). Specifically its activity has been established in serious infections of the female pelvis and genital tract such as endometritis, nongonococcal tubo-ovarian abscess, pelvic cellulitis, and postsurgical vaginal-cuff infection caused by susceptible strains of bacteroides, fusobacteria, peptococci, peptostreptococci, microaerophilic streptococci, or clostridia.* Furthermore, Cleocin PO_4 has proved successful in septicemia and in such nongynecologic conditions as serious intra-abdominal infections (peritonitis, intra-abdominal abscess), serious respiratory-tract infections (empyema, anaerobic pneumonitis, lung abscess), and serious infections of skin and soft-tissue caused by susceptible anaerobic organisms.

Since disc susceptibility testing with current methods is not reliable for anaerobes, *in vitro* dilution methods should be used to determine susceptibility to clindamycin.

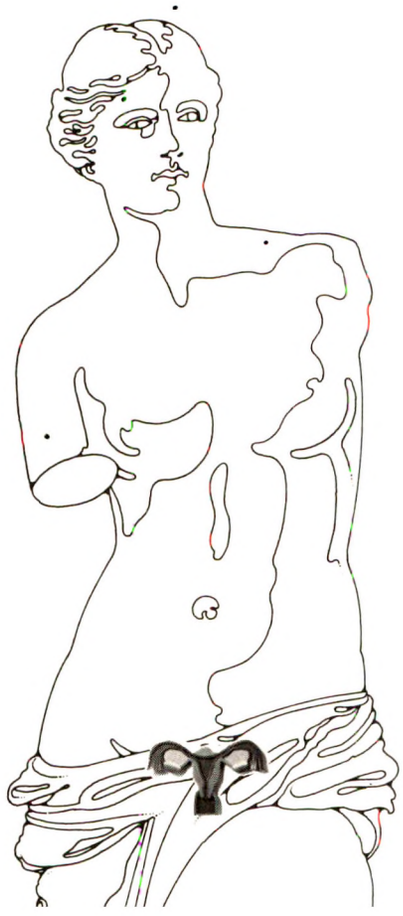
Also of value in serious gram-positive infections The use of Cleocin PO_4 in serious nongynecologic infections caused by susceptible gram-positive organisms has been extensively investigated both bacteriologically and clinically. Because Cleocin PO_4 is a parenteral antibiotic, emphasis has been on evaluation of the drug in serious infections, and the appropriate patients for Cleocin PO_4 are those who are hospitalized with serious illnesses.

Diagnoses have included septicemia and septic shock, acute peritonitis, staphylococcal pneumonia, and massive wound infection. In many cases the infection was secondary to severe accidental trauma, major surgery, or debilitating disease.

Cleocin PO_4 has produced excellent results when the pathogens in these serious infections proved to be staphylococci, streptococci (except enterococci), or pneumococci. With rare exceptions, the organisms proved susceptible *in vitro* and were successfully eradicated from the clinical lesions, regardless of the severity of the infection. Its use in these infections should be reserved for penicillin-allergic patients for whom, in the judgment of the physician, a penicillin is inappropriate.

Other therapeutic considerations:

1) Cleocin PO_4 is clinically effective in serious infections caused by penicillin-resistant or penicillinase-producing staphylococci. However, staphylococcal strains resistant to clindamycin have been encountered, and susceptibility testing should be performed.



2) Cleocin is useful in patients who are sensitive to penicillin. Rare cases of sensitivity to Cleocin resulting in maculopapular rash and urticaria have been reported; as with all antibiotics, it should be used cautiously in atopic patients. Serious anaphylactoid reactions require emergency treatment with epinephrine. Oxygen and intravenous corticosteroids should also be administered as indicated. However, no actual cross-antigenicity with other antibiotics has been demonstrated, *except with lincomycin.*

Because of these two benefits, Cleocin PO_4 can fill a need both for penicillin-sensitive patients and those harboring penicillin-resistant organisms.

Cleocin PO_4 should be reserved for serious infections where less toxic antimicrobial agents are inappropriate, as described in the Indications Section of the brief summary on the following page.

WARNINGS: (See WARNING box on following page.)

If significant diarrhea or colitis occurs during therapy, this antibiotic should be discontinued. (See WARNING box and summary of prescribing information on following page.

Safety for use in pregnancy has not been established.

*Clostridia are more resistant than most anaerobes to clindamycin. Some species, e.g., *C. sporogenes* and *C. tertium,* are frequently resistant to clindamycin.

STERILE SOLUTION

...Cleocin® PO4

clindamycin phosphate injection, Upjohn

WARNING

Clindamycin can cause severe colitis which may end fatally. Therefore, it should be reserved for serious infections where less toxic antimicrobial agents are inappropriate, as described in the Indications Section. It should not be used in patients with nonbacterial infections, such as most upper respiratory tract infections. The colitis is usually characterized by severe, persistent diarrhea and severe abdominal cramps and may be associated with the passage of blood and mucus. Endoscopic examination may reveal pseudomembranous colitis.

When significant diarrhea occurs, the drug should be discontinued or, if necessary, continued only with close observation of the patient. Large bowel endoscopy has been recommended.

Antiperistaltic agents such as opiates and diphenoxylate with atropine (Lomotil®) may prolong and/or worsen the condition.

Diarrhea, colitis, and pseudomembranous colitis have been observed to begin up to several weeks following cessation of therapy with clindamycin.

Each ml contains:

clindamycin phosphate equivalent to	150 mg clindamycin base
benzyl alcohol	5 mg
disodium edetate	0.5 mg
water for injection	qs

When necessary, pH adjusted with NaOH and/or HCl.

Indications: Clindamycin is indicated in the treatment of serious infections caused by susceptible anaerobic bacteria.

Clindamycin is also indicated in the treatment of serious infections due to susceptible strains of streptococci, pneumococci, and staphylococci. Its use should be reserved for penicillin-allergic patients or other patients for whom, in the judgment of the physician, a penicillin is inappropriate. Because of the risk of colitis, as described in the WARNING box, before selecting clindamycin the physician should consider the nature of the infection and the suitability of less toxic alternatives (e.g., erythromycin).

Anaerobes: Serious respiratory tract infections such as empyema, anaerobic pneumonitis and lung abscess; serious skin and soft tissue infections; septicemia; intra-abdominal infections such as peritonitis and intra-abdominal abscess (typically resulting from anaerobic organisms resident in the normal gastrointestinal tract); infections of the female pelvis and genital tract such as endometritis, nongonococcal tubo-ovarian abscess, pelvic cellulitis and postsurgical vaginal cuff infection.

Streptococci: Serious respiratory tract infections; serious skin and soft tissue infections; septicemia; acute hematogenous osteomyelitis.

Pneumococci: Serious respiratory tract infections.

Adjunctive Therapy: In the surgical treatment of chronic bone and joint infections due to susceptible organisms.

Indicated surgical procedures should be performed in conjunction with antibiotic therapy.

Bacteriologic studies should be performed to determine the causative organisms and their susceptibility to clindamycin.

Contraindications: History of hypersensitivity to clindamycin or lincomycin.

Warnings: (See WARNING box.) Mild cases of colitis may respond to drug discontinuance alone. Moderate to severe cases should be managed promptly with fluid, electrolyte and protein supplementation as indicated. Systemic corticoids and corticoid retention enemas may help relieve the colitis. Other causes of colitis should also be considered.

A careful inquiry should be made concerning previous sensitivities to drugs and other allergens. Antagonism has been demonstrated between clindamycin and erythromycin *in vitro*. Because of possible clinical significance, these two drugs should not be administered concurrently.

Usage in Pregnancy: Safety for use in pregnancy has not been established.

Usage in Newborns and Infants: When clindamycin phosphate is administered to newborns and infants, appropriate monitoring of organ system functions is desirable.

Usage in Meningitis: Since clindamycin does not diffuse adequately into the cerebrospinal fluid, the drug should not be used in meningitis.

SERIOUS ANAPHYLACTOID REACTIONS REQUIRE IMMEDIATE EMERGENCY TREATMENT WITH EPINEPHRINE. OXYGEN AND INTRAVENOUS CORTICOSTEROIDS SHOULD ALSO BE ADMINISTERED AS INDICATED.

Precautions: Review of experience to date suggests that a subgroup of older patients with associated severe illness may tolerate diarrhea less well. When clindamycin is indicated in these patients, they should be carefully monitored for change in bowel frequency. Prescribe with caution in individuals with a history of gastrointestinal disease, particularly colitis.



Do not inject intravenously as an undiluted bolus; infuse as directed in package insert. Indicated surgical procedures should be performed in conjunction with therapy. Patients with very severe renal disease and/or very severe hepatic disease accompanied by severe metabolic aberrations should be dosed with caution and serum clindamycin levels monitored during high dose therapy.

Prescribe with caution in atopic individuals. During prolonged therapy, periodic liver and kidney function tests and blood counts should be performed. Use may result in overgrowth of non-susceptible organisms, particularly yeast. Should superinfection occur, adjust therapy as clinical situation dictates. Clindamycin has been shown to have neuromuscular blocking properties that may enhance the action of other neuromuscular blocking agents. Use with caution in patients receiving such agents.

Adverse Reactions: *Gastrointestinal:* Abdominal pain, nausea, vomiting and diarrhea. (See WARNING box.) *Hypersensitivity Reactions:* Maculopapular rash and urticaria have been observed. Generalized mild to moderate morbilliform-like skin rashes are the most frequently reported of all adverse reactions. Rare instances of erythema multiforme, some resembling Stevens-Johnson syndrome, have been associated with clindamycin. A few cases of anaphylactoid reactions have been reported. If a hypersensitivity reaction occurs, the drug should be discontinued. The usual agents (epinephrine, corticosteroids, antihistamines) should be available for emergency treatment of serious reactions. *Liver:* Jaundice and abnormalities in liver function tests have been observed during clindamycin therapy. *Hematopoietic:* Neutropenia, eosinophilia, agranulocytosis and thrombocytopenia have been reported; no direct etiologic relationship to concurrent clindamycin therapy has been made. *Local Reactions:* Pain, induration and sterile abscess have been reported after intramuscular injection and thrombophlebitis after intravenous infusion. Reactions can be minimized or avoided by giving deep intramuscular injections and avoiding prolonged use of indwelling intravenous catheters.

How Supplied: Available as sterile solution with each ml containing clindamycin phosphate equivalent to 150 mg clindamycin base. Ampoules of 2 and 4 ml.

Caution: Federal law prohibits dispensing without prescription.

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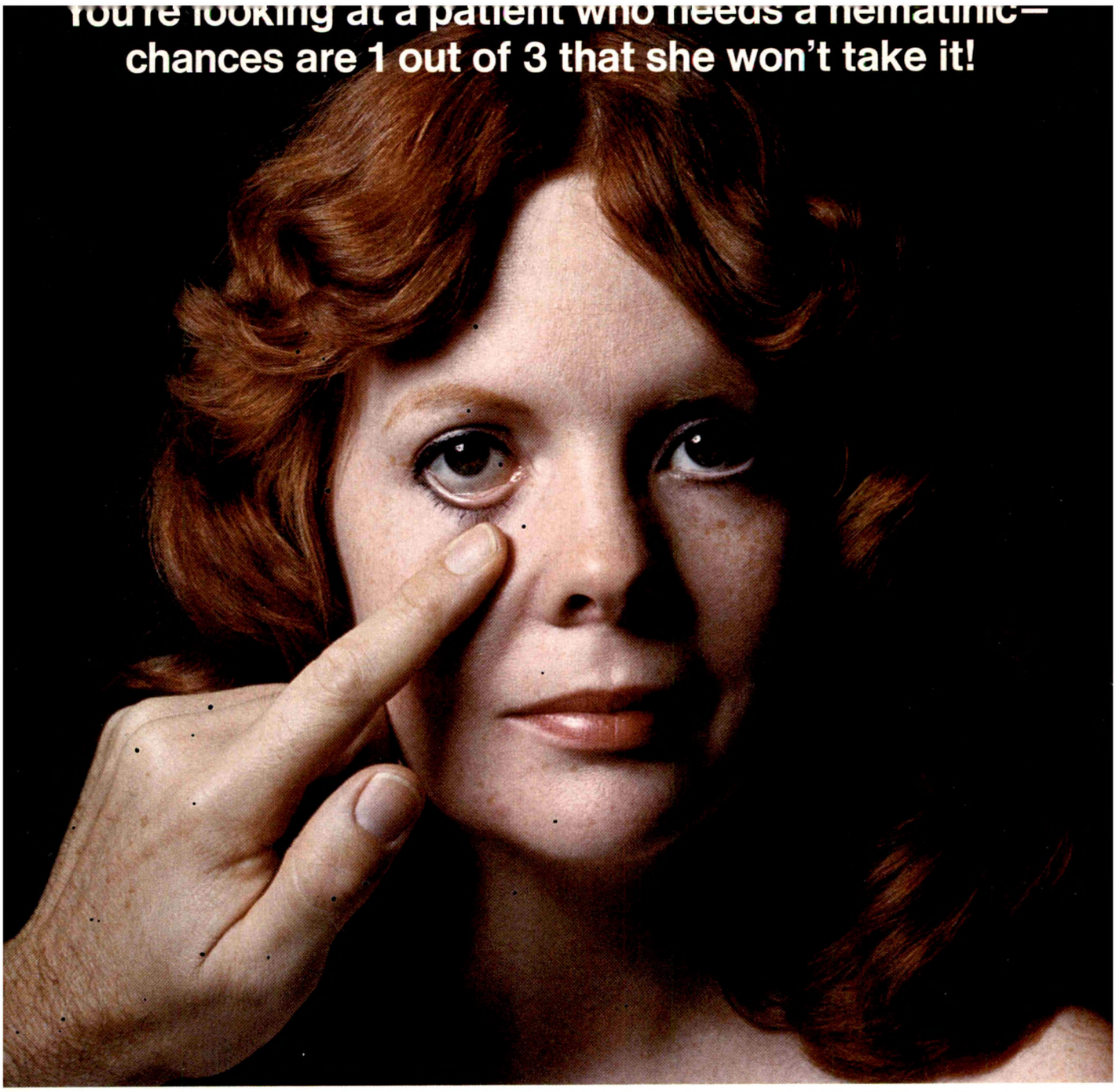
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1. Bonnar, J., et al., Lancet, 1:457, 1969.
2. Porter, A. M. W., Brit. Med. J., 1:218, 1969.

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INDICATIONS: For conditions in which iron deficiency occurs concomitantly with deficient intake or increased need for the B-complex vitamins (contains no folic acid).

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WARNINGS: Folic acid alone is improper therapy in the treatment of pernicious anemia and other megaloblastic anemias where vitamin B_{12} is deficient.

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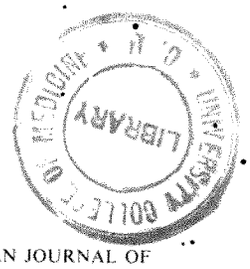
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Each green tablet in the 28-day regimen contains inert ingredients.

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Description: Each white ORTHO-NOVUM 1/80 tablet contains 1 mg of the progestational substance, norethindrone, together with 0.08 mg of the estrogenic compound, mestranol. Each green tablet in the 28-day regimen contains inert ingredients.

Actions: ORTHO-NOVUM 1/80□21 and ORTHO-NOVUM 1/80□28 act through the mechanism of gonadotrophin suppression, i.e., through the estrogenic and progestational actions of the ingredients. Alterations in cervical mucus and in the endometrium may also contribute to the efficacy of ORTHO-NOVUM 1/80□21 and ORTHO-NOVUM 1/80□28.

Special note: Oral contraceptives have been marketed in the United States since 1960. Reported pregnancy rates vary from product to product. The effectiveness of the sequential products appears to be somewhat lower than that of the combination products. Both types provide almost completely effective contraception.

The physician should consider a thorough discussion with each patient about the prescribing information, risks and benefits associated with oral contraceptives, taking into account various factors such as age and other physical conditions.

An increased risk of thromboembolic disease associated with the use of hormonal contraceptives has now been shown in studies conducted in both Great Britain and the United States. Other risks, such as those of elevated blood pressure and reduced tolerance to carbohydrates, have been reported and appropriate tests should be conducted to monitor these during oral contraceptive therapy. Liver disease has also been reported, and the physician should be alert to its earliest manifestations.

Long-term administration of both natural and synthetic estrogens in subprimate animal species in multiples of the human dose increases the frequency for some animal carcinomas. These data cannot be transposed directly to man. The possible carcinogenicity due to the estrogens can neither be affirmed nor refuted at this time. Close clinical surveillance of all women taking oral contraceptives must be continued.

Indication: Contraception.

Contraindications: 1. Thrombophlebitis, thromboembolic disorders, cerebral vascular disease, myocardial infarction, or a past history of these conditions. 2. Markedly impaired liver function. 3. Known or suspected carcinoma of the breast. 4. Known or suspected estrogen-dependent neoplasia. 5. Undiagnosed abnormal genital bleeding. 6. Known or suspected pregnancy.

Warnings: 1. The physician should be alert to the earliest manifestations of thrombotic and thromboembolic disorders, thrombophlebitis, cerebrovascular disorders including hemorrhage, pulmonary embolism and retinal thrombosis. Should any of these occur or be suspected, the drug should be discontinued immediately.

Retrospective studies of morbidity and mortality in Great Britain and studies of morbidity in the United States have shown a statistically significant association between thrombophlebitis, pulmonary embolism and cerebral vascular disease, occlusive or hemorrhagic, and the use of oral contraceptives. There have been three principal studies in Great Britain[1-3] leading to these conclusions and three in this country.[4-7] The estimate of the relative risk of thromboembolism in the study by Vessey and Doll[3] was about sevenfold, while the United States studies found relative risks of 4.4 to 11, meaning that the users are several times as likely to undergo thromboembolic disease without evident cause as nonusers.

In May, 1974, the Royal College of General Practitioners in England[8] issued an interim report of its continuing large-scale prospective study comparing a user group to a nonuser group. This study in its interim analysis states, "A statistically significant higher rate of reporting of cerebrovascular accidents in Takers is evident, but the numbers are too small to justify an estimation of the degree of risk." The study also reported a higher incidence of superficial and deep vein thrombosis in users as compared to nonusers. The risk of superficial and deep vein thrombosis was reported to be lower in women using 50 mcg estrogen preparations.

The Sartwell study[4] indicated that the risk did not persist after discontinuation of administration. Both the Sartwell and the Royal College studies indicated that the degree of risk was not associated with duration of treatment.

In a collaborative American study[5,6] of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the relative risk of thrombotic stroke was 4.1 to 9.5 times greater in users than in nonusers. A comparable estimate for hemorrhagic stroke was 2.0.

None of the American studies was designed to evaluate a difference between products. However, the Sartwell study[4] suggested that there might be an increased risk of thromboembolic disease in users of sequential products.

Other retrospective studies[9,10] have reported an increased risk of post-surgery thromboembolic complications in oral contraceptive users. It has been recommended that therapy be discontinued at least one month prior to elective surgery.

In May of 1975 two separate retrospective case control studies relating to fatal and nonfatal myocardial infarction in users and nonusers of oral contraceptives were reported.[11,12] The studies estimate that the increased risk of hospital admission for myocardial infarction associated with the use of oral contraceptives as compared to nonusers is 2.7 times in the 30-39 age group and 5.7 times in the 40-44 age group; and the risk of death associated with the use of oral contraceptives as compared to nonusers was increased 2.8 times for users in the 30-39 age group and 4.7 times in the 40-44 age group. One of these studies reported that oral contraceptive users who had myocardial infarction were more likely to be heavy smokers and have certain additional risk factors, i.e., treated hypertension and diabetes, pre-eclamptic toxemia, obesity and type II hyperlipoproteinemia.

2. Discontinue oral contraceptive medication if there is: gradual or sudden partial or complete loss of vision; proptosis or diplopia; onset or aggravation of migraine or development of headache of a new pattern which is recurrent, persistent or severe; papilledema; or any evidence of retinal vascular lesions.

3. Fetal abnormalities have been reported to occur in the offspring of women who have taken progestogens and/or estrogens during pregnancy.[13,14] The safety of ORTHO-NOVUM 1/80□21 or ORTHO-NOVUM 1/80□28 in pregnancy has not been demonstrated. Pregnancy should be ruled out before initiating or continuing the contraceptive regimen. Pregnancy should always be considered if withdrawal bleeding does not occur.

4. A small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The long-range effect to the nursing infant cannot be determined at this time.

5. Hepatic lesions (adenomas, hepatomas, hamartomas, regenerating nodules, etc.), occasionally fatal, have been reported in women on oral contraceptives. Such lesions may present as an abdominal mass or with the signs and symptoms of an acute abdomen. These lesions should be considered if the patient has abdominal pain or evidence of intra-abdominal bleeding. This has been reported in short-term as well as long-term users of oral contraceptives.

Precautions: 1. A thorough history and physical examination should be performed before prescribing oral contraceptives and periodically during their administration and should include special reference to breasts and pelvic organs, including Papanicolaou smear since estrogens have been known to produce tumors, some of them malignant, in five species of subprimate animals. 2. Endocrine and possibly liver function tests may be affected by treatment with ORTHO-NOVUM 1/80□21 or ORTHO-NOVUM 1/80□28. Therefore, if such tests are abnormal in a patient taking ORTHO-NOVUM 1/80□21 or ORTHO-NOVUM 1/80□28, it is recommended that they be repeated after the drug has been withdrawn for two months. 3. Under the influence of estrogen-progestogen preparations, pre-existing uterine fibromyomata may increase in size. 4. Because these agents may cause some degree of fluid retention, conditions which might be influenced by this factor, such as epilepsy, migraine, asthma, cardiac or renal dysfunction, require careful observation. 5. In breakthrough bleeding, and in all cases of irregular bleeding per vaginam, nonfunctional causes should be borne in mind. In undiagnosed bleeding per vaginam, adequate diagnostic measures are indicated. 6. Patients with a history of psychic depression should be carefully observed and the drug discontinued if the depression recurs to a serious degree. 7. Any possible influence of prolonged ORTHO-NOVUM 1/80□21 or ORTHO-NOVUM 1/80□28 therapy on pituitary, ovarian, adrenal, hepatic or uterine function awaits further study. 8. A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. The mechanism of this decrease is obscure. For this reason, diabetic patients should be carefully observed while receiving ORTHO-NOVUM 1/80□21 or ORTHO-NOVUM 1/80□28 therapy. 9. The age of the patient constitutes no absolute limiting factor, although treatment with ORTHO-NOVUM 1/80□21 or ORTHO-NOVUM 1/80□28 may mask the onset of the climacteric. 10. The pathologist should be advised of ORTHO-NOVUM 1/80□21 or ORTHO-NOVUM 1/80□28 therapy when relevant specimens are submitted. 11. Susceptible women may experience an increase in blood pressure following administration of contraceptive steroids. 12. Cholestatic jaundice has been reported in users of oral contraceptives. If this occurs ORTHO-NOVUM 1/80□21 or ORTHO-NOVUM 1/80□28 should be discontinued. This condition is more likely to occur in patients who have experienced cholestatic jaundice of pregnancy. Patients with a history of cholestatic jaundice of pregnancy should be carefully observed during ORTHO-NOVUM 1/80□21 or ORTHO-NOVUM 1/80□28 therapy.

Adverse reactions observed in patients receiving oral contraceptives: A statistically significant association has been demonstrated between use of oral contraceptives and the following serious adverse reactions: thrombophlebitis, pulmonary embolism, cerebral thrombosis and hemorrhage, myocardial infarction, gallbladder disease.

Although available evidence is suggestive of an association, such a relationship has been neither confirmed nor refuted for the following serious adverse reactions: neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis, hepatic lesions with or without intra-abdominal bleeding.

The following adverse reactions are known to occur in patients receiving oral contraceptives: nausea, vomiting, gastrointestinal symptoms (such as abdominal cramps and bloating), breakthrough bleeding, spotting, change in menstrual flow, amenorrhea during and after treatment, edema, chloasma or melasma, which may persist, cholestatic jaundice, migraine, rash (allergic), mental depression, change in weight (increase or decrease), breast changes (tenderness, enlargement and secretion), changes in cervical erosion and cervical secretions, suppression of lactation when given immediately post-partum, rise in blood pressure in susceptible individuals.

Although the following adverse reactions have been reported in users of oral contraceptives, an association has been neither confirmed nor refuted: anovulation post-treatment, which tends to occur more frequently in women with a history of menstrual disorders, premenstrual-like syndrome, changes in libido, changes in appetite, cystitis-like syndrome, headache, intolerance to contact lenses, nervousness, dizziness, fatigue, backache, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruptions, itching, vaginitis.

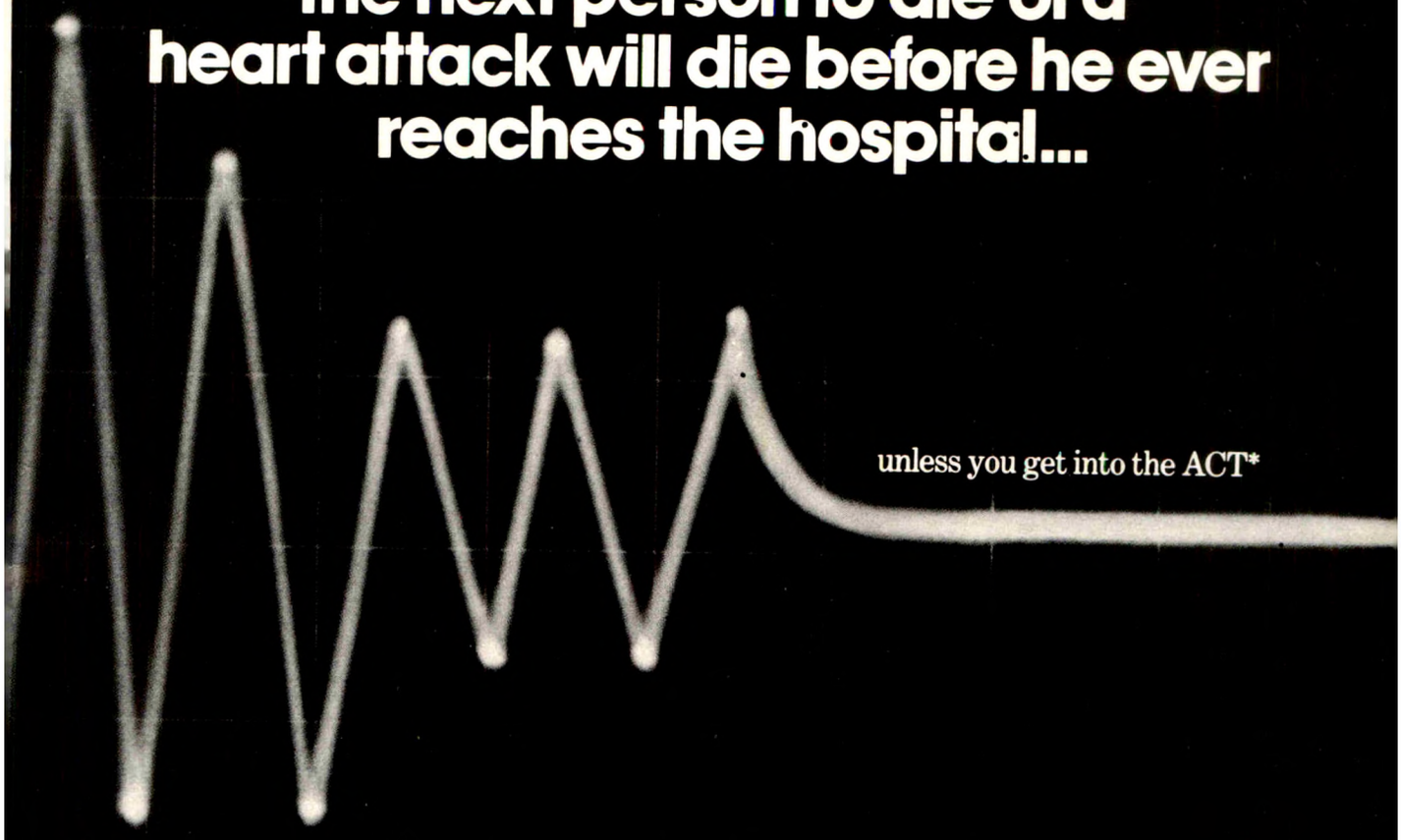
The following laboratory results may be altered by the use of oral contraceptives: hepatic function—increased sulfobromophthalein retention and other tests; coagulation tests—increase in prothrombin, Factors VII, VIII, IX and X; decrease in anti-thrombin III; increase in platelet aggregability; thyroid function—increase in PBI, and butanol extractable protein-bound iodine and decrease in T^3 uptake values; metyrapone test; pregnanediol determination.

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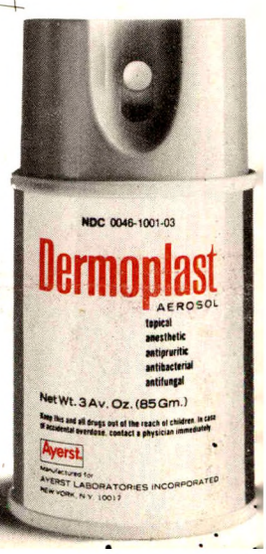
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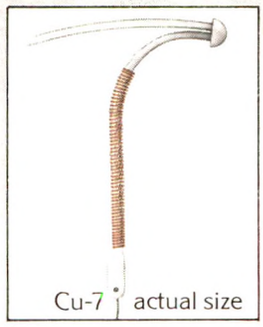
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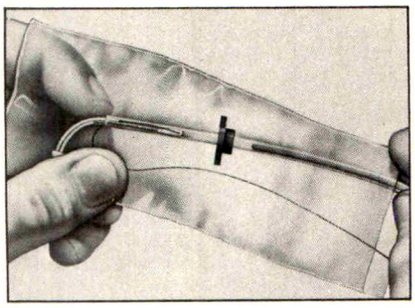
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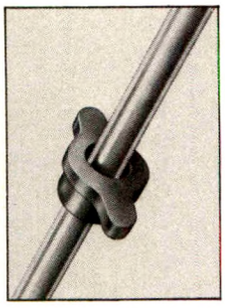


Cu-7®
intrauterine copper contraceptive

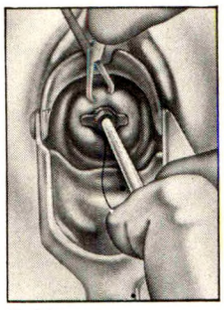
How five newly designed features make it easier to use Cu-7® intrauterine copper contraceptive.



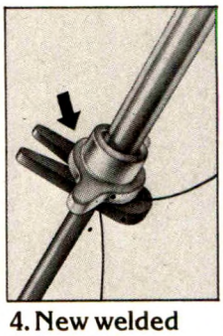
1. New sterile sheath
Helps maintain sterility during loading procedure and cervical-stop setting.



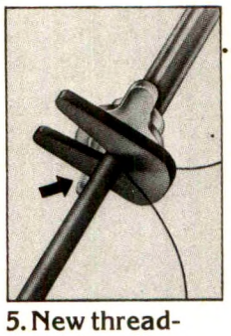
2. New blue-winged cervical stop
A visual guide to aid in correct positioning of the "seven" in the uterus.



3. New flexible rod
Facilitates insertion of "seven" into virtually any normal uterine cavity in seconds.



4. New welded tube-stop
Serves as handle for withdrawing the tube and removal of instrument.



5. New thread-retaining clip
When held to tube-stop during procedure, facilitates insertion and makes tube-crimping virtually impossible.

Cu-7 (brand of intrauterine copper contraceptive)

Description: The plastic component of the Cu-7 is composed of pharmaceutical grade polypropylene homopolymer with barium sulfate added to render it radiopaque. Its shape approximates the number 7; it is substantially smaller than previously available intrauterine devices.

Coiled around the vertical limb is 89 mg. of pure, virgin electrolytic copper wire providing 200mm of exposed copper surface area. A polypropylene retrieval thread is fastened to the free end of the vertical limb of the Cu-7.

The Cu-7 is supplied with a simple tubular polypropylene inserter. All components are sterile.

Action: Available data indicate that the contraceptive effectiveness of the Cu-7 is enhanced by a minute quantity of copper released continuously from the copper coil into the uterine cavity. The exact mechanism by which metallic copper enhances the contraceptive effect of an IUD has not been conclusively demonstrated. Various hypotheses have been advanced, the most common being that copper placed in the uterus interferes with enzymatic or other processes that regulate blastocyst implantation.

Animal studies suggest that copper may play an additional role by reduction of sperm transport within the uterine environment.

Indication: The Cu-7 is indicated for contraception.

Contraindications: The presence of any of the following, at the time of insertion, constitutes a contraindication: pregnancy, abnormalities of the uterine cavity, acute pelvic inflammatory disease or a history of repeated pelvic inflammatory disease, postpartum endometritis or infected abortion in the past three months, endometrial disease such as hyperplasia or carcinoma and a known or suspected allergy to copper.

Warnings: Recent reports suggest an increased incidence of septic abortion associated in some instances with septicemia, septic shock and death in patients becoming pregnant with an IUD in place. In some cases, initial symptoms have been insidious and not easily recognized. If pregnancy should occur with a Cu-7 in situ, the Cu-7 should be removed if the string is visible or, if removal proves to be or would be difficult, interruption of the pregnancy should be considered and offered as an option. If the patient elects to maintain the pregnancy and the Cu-7 remains in situ, she should be warned of the suggested incidence of increased risk of sepsis and followed with close vigilance.

The long-term effects on the offspring of the presence of copper in the uterus during pregnancy are unknown.

Perforations of the uterus have occurred. In this case the device should be removed immediately.

The use of microwave therapy in patients with metal prosthetics may cause heat injury to the surrounding tissue. Therefore, microwave therapy to the abdominal and sacral areas should not be used on patients wearing a Cu-7.

Additional amounts of copper available to the body from the Cu-7 may precipitate symptoms in women with undiagnosed Wilson's disease, the incidence of which is one in two hundred thousand.

Precautions: A pelvic examination should be done.

Sound the uterus prior to insertion and exercise care to avoid perforation. Do not use excessive force. The possibility of insertion in the presence of an existing undetermined pregnancy is reduced if insertion is performed shortly following a menstrual period. The Cu-7 should not be inserted post partum or post abortion until involution of the uterus has been completed.

Occasional perforations of the uterus (.03%) have been reported, usually during insertion into patients less than two months post abortion or post partum. If penetration into abdominal cavity occurs, laparotomy should be performed and the device recovered. Local inflammatory reaction with abscess formation is a possibility if a device is left in the abdomen.

If any patient with a Cu-7 suddenly develops overt clinical hepatitis or abnormal liver function tests, appropriate diagnostic procedures should be initiated.

The Cu-7 should be removed if pelvic infection which is unresponsive to treatment occurs.

The device should be replaced every two years. There is no evidence of decreasing effectiveness with time before two years, but the contraceptive effectiveness of the Cu-7 after two years has yet to be established.

The patient should be told that some light bleeding or cramps may occur during the first few days after insertion, but if these symptoms continue or are severe she should report to her physician. She should be instructed on how to inspect periodically to make certain that the thread still protrudes from the cervix and cautioned that there is no protection if the device has been expelled. She should also be cautioned not to pull on the thread and displace the Cu-7 and told to return in two years for its replacement.

A report has appeared in the literature suggesting that a copper-induced urticarial allergic skin reaction developed in a woman wearing a copper IUD. If symptoms of such an allergic response occur the patient should be instructed to tell the consulting physician that a copper-bearing device is being worn.

Adverse Reactions: Perforation of the uterus has occurred.

The incidence of spontaneous abortion, when conception occurs with intrauterine devices in situ, appears to be increased over that in unprotected women.

Insertion cramping, usually of no more than a few seconds' duration, may occur; however, some women may experience residual cramping for several hours or even days. Transient spotting or bleeding or prolongation of menstrual flow may occur in the first few cycles. Uncommonly, pelvic infection has been reported. Complete or partial expulsion may sometimes occur, particularly in those patients with uteri measuring less than 6.5 cm. by sounding. The following complaints have also been reported, although their relation to the Cu-7 has not been established: amenorrhea or delayed menses, backaches, cervical erosion, cystic masses in pelvis, vaginitis, leg pain or soreness, weight loss or gain, nervousness, dyspareunia, and cystitis.

Dosage and Administration: A single Cu-7 is to be inserted into the uterine cavity (see Precautions section). The small diameter of the Cu-7 enables easy insertion into the nulliparous as well as the multiparous uterus. Present information indicates that the efficacy is retained for at least 24 months. Until accurate data indicating a longer effective life become available, the Cu-7 should be removed and a new one inserted on or before 24 months from the date of insertion. If partial expulsion occurs, removal is indicated, and a new Cu-7 may be inserted. Removal of the Cu-7 may also be indicated in the event of heavy or persistent bleeding.

How Supplied: Available in cartons containing 6 or 30 sterile units (Cu-7 with an inserter) and sufficient patient identification cards.

Clinical Studies: Different event rates have been recorded with the use of different intrauterine contraceptive devices. Inasmuch as these rates are usually derived from separate studies conducted by different investigators in several population groups, they cannot be compared with precision. Furthermore, event rates tend to be lower as clinical experience is expanded, possibly due to retention in the clinical study of those patients who accept the treatment regimen and did not discontinue due to adverse reactions or pregnancy. In clinical trials of the Cu-7 conducted by Searle Laboratories, use effectiveness was determined as follows for parous and nulliparous women, as tabulated by the life table method. (Rates are expressed as events per 100 women through 12 and 24 months of use.) This experience encompasses 305,958 woman-months of use, including 12 months for 9,549 women and 24 months for 7,425.

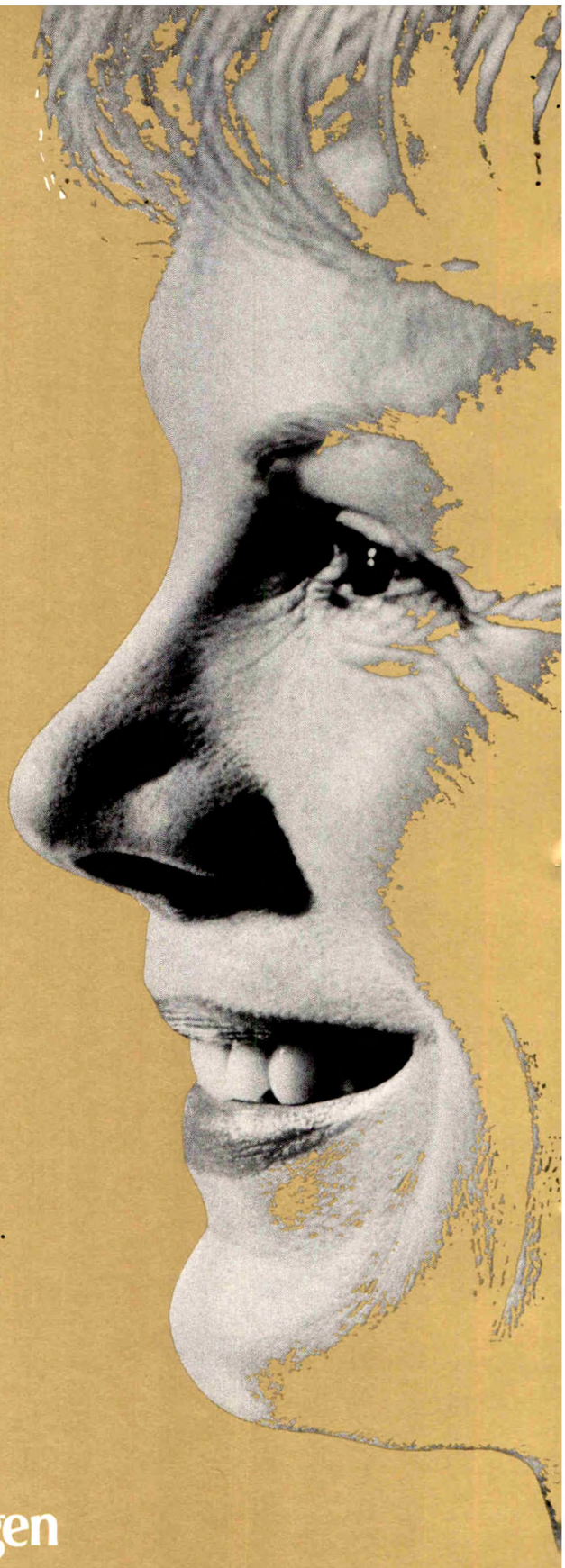
	12 Months		24 Months	
	Parous	Nulliparous	Parous	Nulliparous
Pregnancy	1.6	1.2	2.4	2.0
Expulsion	5.4	7.7	6.4	8.7
Medical Removal	9.3	12.3	14.9	17.2
Continuation Rate	78.7	73.2	65.0	60.5

Please see complete product labeling for insertion and removal techniques.

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6B1-R

Estrogen replacement therapy moves forward



the major human estrogen micronized for oral effectiveness

Estrace®
(estradiol)

1-mg. and 2-mg. scored tablets

- not esterified, not conjugated
- estradiol...the active estrogen at the cellular level

from Mead Johnson

overall, 95% of patients studied gained adequate or complete relief of their symptoms[1]

Estrace (estradiol) was given to 369 estrogen-deficient women for periods ranging up to a year or more (average of six months); 319 of these women received an initial dosage of 1 or 2 mg. per day and were evaluated for efficacy. *Complete disappearance* of hot flushes was experienced by 227 (76%) of 298 women, of sweating by 131 (80%) of 164 women, of tingling by 62 (94%) of 66 women, and of genital atrophy by 44 (88%) of 50 women. In general, 95% of the patients evaluated experienced either adequate or complete relief of their symptoms. Remarkably, in 77% of the patients, symptoms were controlled without need to adjust the initial dosage—usually 2 mg. daily. (Dosage after adjustment ranged from 1 mg. to 3 or 4 mg. a day.)[1]

cyclic therapy recommended

Recently, published reports of retrospective human data have emphasized that patients using estrogens may have an associated increased risk of endometrial cancer. This information makes cyclic rather than continuous estrogen replacement therapy even more prudent. (See brief summary.)

With Estrace, treatment should be initiated with a dose of 1 or 2 mg. daily and then adjusted to the lowest dose possible still maintaining the relief of symptoms. The usual regimen for cyclic administration of Estrace is three weeks on drug, followed by one week off drug. Some patients may require a slightly shorter estrogen-free interval (4–6 days) to prevent recurrence of menopausal symptoms.

estradiol... orally effective

The technique of micronization has opened up the oral route to natural human estrogen replacement with Estrace. Reduced particle size of the estradiol in Estrace makes effective gastrointestinal absorption possible. Onset of action is rapid, with marked increases in levels of circulating estradiol and estrone; concomitant decreases in serum concentrations of FSH and LH are sustained over a 24-hour period.[2]

Because estradiol can be assayed in plasma, Estrace can provide objective[2] as well as clinical evidence[1] of ready availability and biologic activity.

well accepted by patients[1]

In the 369 patients previously described, the incidence of estrogen-induced side effects with Estrace was relatively low. Incidence of nausea was remarkably low—only 2% of patients before dosage adjustment. Some patients who had complained of this problem while on conjugated equine estrogens reported its complete disappearance on changing to Estrace. Whereas before dosage adjustment 24% experienced breast tenderness, 31% edema, 18% (of 151 with intact uteri) bleeding, and 4% weight gain, only about 2% of the 369 patients dropped out because of side effects.[1]

long-term therapy should be monitored

A few instances of significant gynecologic disease became apparent during clinical investigations.[1] Endometrial hyperplasia was discovered in seven patients, all of whom had previously received estrogen therapy for one to three years prior to entering the study. One patient was reported to have carcinoma *in situ* and another showed adenocarcinoma of the endometrium. In clinical practice, the physician should be alerted to the potential of thrombotic disorders and be prepared to discontinue medication immediately if they occur. Because of variations in normal endogenous hormone production, certain patients may exhibit signs of excessive estrogenic stimulation. Adverse reactions generally associated with estrogen administration may occur and these are listed in the brief summary. Prior to initiation of treatment with Estrace or any other estrogenic preparation, an adequate physical examination should be given.

References

1. Callantine, M.R., *et al.*: Micronized 17β-estradiol for oral estrogen therapy in menopausal women, Obstet. Gynecol. *46*:37–41 (July) 1975.
2. Yen, S.S.C., *et al.*: Circulating estradiol, estrone and gonadotropin levels following the administration of orally active 17β-estradiol in postmenopausal women, J. Clin. Endocrinol. Metab. *40*:518–521 (March) 1975.

For Brief Summary, please see following page.

Estrogen replacement therapy moves forward...

Estrace®
(estradiol)

the major human estrogen micronized for oral effectiveness



Indications: Estrace® (17β-Estradiol) is indicated for relief of endogenous estrogen deficiency and associated menopausal symptoms.

Contraindications: Estrace® is contraindicated in patients with 1) markedly impaired liver function; 2) known or suspected carcinoma of the breast, except those cases of progressing disease not amenable to surgery or irradiation occurring in women who are at least 5 years postmenopausal, or in men in whom castration is not feasible; 3) known or suspected estrogen dependent neoplasia, such as carcinoma of the endometrium; 4) undiagnosed abnormal genital bleeding; 5) thrombophlebitis, thromboembolic disorders, cerebral apoplexy, or with a past history of these conditions; and 6) pregnancy.

Warnings: The physician should be alert to the earliest manifestations of thrombotic disorders (thrombophlebitis, cerebrovascular disorders, pulmonary embolism and retinal thrombosis). If these occur or are suspected the drugs should be discontinued immediately. Discontinue medication pending examination if there is a sudden onset of proptosis, diplopia, or migraine. If examination reveals papilledema or retinal vascular lesions, medication should be withdrawn. Estrogens may be excreted in the mother's milk and an estrogenic effect upon the infant has been described. The long range effect on the nursing infant cannot be determined at this time. Hypercalcemia may occur in as many as 15% of breast cancer patients with metastases and this usually indicates progression of bone metastases. This occurrence depends neither on dose nor on immobilization. In the presence of untoward effects such as progression of the cancer or hypercalcemia, estrogen medication should be stopped. A statistically significant association has been reported between maternal ingestion during pregnancy of diethylstilbestrol and the occurrence of vaginal carcinoma developing years later in the offspring. Whether such an association is applicable to all estrogens is not known at this time. In any event, estrogens are not indicated for use during pregnancy.

Precautions: Because normal endogenous hormone production varies individually, certain patients may be unusually responsive to estrogenic therapy and may respond with undesirable manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding, mastodynia, edema, etc. Because of estrogen induced salt and water retention, these drugs should be used with caution in patients with epilepsy, migraine, asthma, cardiac or renal disease. Patients with a history of psychic depression should be carefully observed and the drug discontinued if the depression recurs to a serious degree. In the event that any unexplained or excessive vaginal bleeding would occur while on estrogen therapy, nonfunctional causes should be borne in mind. The drug should be discontinued and a thorough investigation made as to the cause, being certain to rule out the possibility of malignancy. Pre-existing uterine fibromyomata may increase in size while using this product, therefore, patients should be examined at regular intervals while receiving estrogenic therapy. Women with a strong family history of cancer, recurrent chronic cystic mastitis, or abnormal mammograms should be administered estrogens with caution. Because of a possible decrease in glucose tolerance, diabetic patients should be followed closely. Because estrogens influence the metabolism of calcium and phosphorus, they should be used with caution in patients with certain metabolic bone diseases that are associated with hypercalcemia or in patients with renal insufficiency. The pathologist should be advised of estrogen therapy when relevant specimens are submitted. Because of the effects of estrogens on epiphyseal closure, they should be used judiciously in young patients in whom bone growth is not complete. A pre-treatment physical examination should include special reference to breast and pelvic organs as well as a Papanicolaou smear since estrogens have been known to produce tumors, some of them malignant, in five species of animals. When large doses of estrogens are used, urinary stress incontinence may occur in nonpregnant females. Prolonged high doses of estrogens will inhibit anterior pituitary functions. This should be borne in mind when treating patients in whom fertility is desired. Continuous use of estrogens will result in prolonged stimulation of the endometrium and breast. In order to avoid this, oral estrogens should be administered cyclically in the menopausal or hypogonadal patient. The age of the patient constitutes no absolute limiting factor, although treatment with estrogens may mask the onset the climacteric. Certain endocrine and liver function tests may be affected by treatment with estrogens. If such tests are abnormal in a patient taking these drugs it is recommended that they be repeated after the drug has been withdrawn for two months. Any possible influence of prolonged estrogen therapy pituitary, ovarian, adrenal, hepatic, or uterine function awaits further study.

Adverse Reactions: A statistically significant association has been demonstrated between use of estrogen-progestin oral contraceptives and the follow serious reactions: Thrombophlebitis, pulmonary embolism, and cerebral thro bosis. Although available evidence is suggestive of an association, such a rel tionship has been neither confirmed nor refuted for the following serious rea tions: coronary thrombosis and neuro-ocular lesions (e.g. retinal thrombosis and optic neuritis). The following adverse reactions are known to occur in patients receiving estrogens: nausea, vomiting, anorexia, gastrointestinal symptoms (such as abdominal cramps or bloating), edema, breakthrough ble ing, spotting or withdrawal bleeding, breast tenderness and enlargement, cha in body weight (increase or decrease), headache, increased cervical mucus, allergic rash, loss of libido and gynecomastia in the male, reactivation of endometriosis, aggravation of migraine headaches, hepatic cutaneous porphyri becoming manifest, cholestatic jaundice, rise in blood pressure in susceptibl individuals, mental depression, cystitis-like syndrome, loss of scalp hair, erythema nodosum, hemorrhagic eruption, premenstrual-like syndrome, changes in libido, changes in appetite, nervousness, dizziness, fatigue, backache, erythema multiforme, itching, possible diminution in lactation when giv immediately post-partum, irritability, and malaise.

Important Notes: An increased risk of thromboembolic disease associated w the use of oral contraceptives containing estrogens and progestins has now been conclusively established. Retrospective studies have shown a statistical significant association between thrombophlebitis, pulmonary embolism, and cerebral thrombosis and embolism and the use of these drugs. There have bee three principal studies in Great Britain[1-3] and one in the United States[4] leading this conclusion. As a result of these studies, it has been estimated that users oral contraceptives containing estrogens are 4 to 7 times more likely than non users to develop thromboembolic disease without evident cause. The America study also indicated that the increased risk did not persist after discontinuati nor was it enhanced by long continued administration. Although the American study was not designed to evaluate a difference between products, it did sugg that there might be an increased risk of thromboembolic disease in users of se quential products. Confirmation of this finding requires further study. In a mo recent analysis of data derived from several national adverse reaction reportin systems,[5] British investigators concluded that the risk of thromboembolism, i cluding coronary thrombosis, is directly related to the dose of estrogen used i oral contraceptive products. Their analysis did suggest, however, that the qua tity of estrogen may not be the sole factor involved. Nevertheless, in view of th study, as well as others that have demonstrated a positive relationship betwee estrogens and thromboembolism, it would seem prudent and in keeping with basic therapeutic principles, to utilize, whenever feasible, the smallest effectiv dose of estrogen in treating patients. Risks associated with certain other know adverse reactions, such as elevated blood pressure, liver dysfunction, and reduced tolerance to carbohydrate, have not as yet been quantitated. Long ter administration of both natural and synthetic estrogens in subprimate animal species in multiples of the human dose increases the frequency for some anim carcinomas. These data cannot be transposed directly to man. The possible ca cinogenicity due to the estrogens can neither be confirmed nor refuted at this time. Close clinical surveillance of all women taking estrogens must be continued. It has been reported in a recent study done in the United States[6] that th maternal ingestion of diethylstilbestrol during pregnancy appears to increase risk of vaginal adenocarcinoma developing years later in the offspring exposed

(1) Royal College of General Practitioners: Oral Contraception and Thrombo-Embolic Disease. J. Coll. Gen. Pract. 13:267-279. 1967. (2) Inman, W.H.W. and Vessey, M.P., Investigation of Deaths from Pulmonary, Coronary and Cerebral Thrombosis and Embolism in Women in Childbearing Age. Brit. Med. J., 2:193-199, 1968. (3) Vessey, M.P. and Doll, R., Investigation of Relation Between Use of Oral Contraceptives and Thromboembolic Disease: A Further Report. Brit. Med. J., 2:651-657, 1969. (4) Sartwell, P.E., Masi, A.T., Arthes, F.G., Greene, G.R., and Smith, H.E., Thromboembolism and Oral Contraceptives: An Epidemiological Case-Control Study. Am. J. Epicem. 90:365-380, (November) 1969. (5) Inman, W.H.W., Vessey, M.P., Westerholm, B., Engelund, A., Thromboembolic Disease and the Steroidal Content of Oral Contraceptives. Brit. Med. J., 25th April, 1970. (6) Herbst, et al. — Adenocarcinoma of the Vagina — N. England Journal of Medicine, Vol. 284, Number 16 (April 22, 1971).

Dosage and Administration: Treatment is usually initiated with a dose of 1 o 2 mg. daily of micronized estradiol. Dosage may be adjusted to control the presenting symptoms depending on the response of the individual patient; the minimal effective dose for maintenance therapy should be determined by titration. To avoid continuous stimulation of the reproductive tract and breasts, cyclic therapy is recommended. The usual regimen consists of three weeks on drug followed by one week off drug. Some patients may require a slightly short estrogen-free interval (4-6 days) to prevent recurrence of menopausal symptoms. Withdrawal bleeding of unusual amount or duration should be investigated.

How Supplied: Estrace® 1 mg; lavender scored tablets, NDC 0087-0755-01, bottles of 100. Estrace® 2 mg; turquoise scored tablets, NDC 0087-0756-01, bottles of 100.

Mead Johnson LABORATORIES

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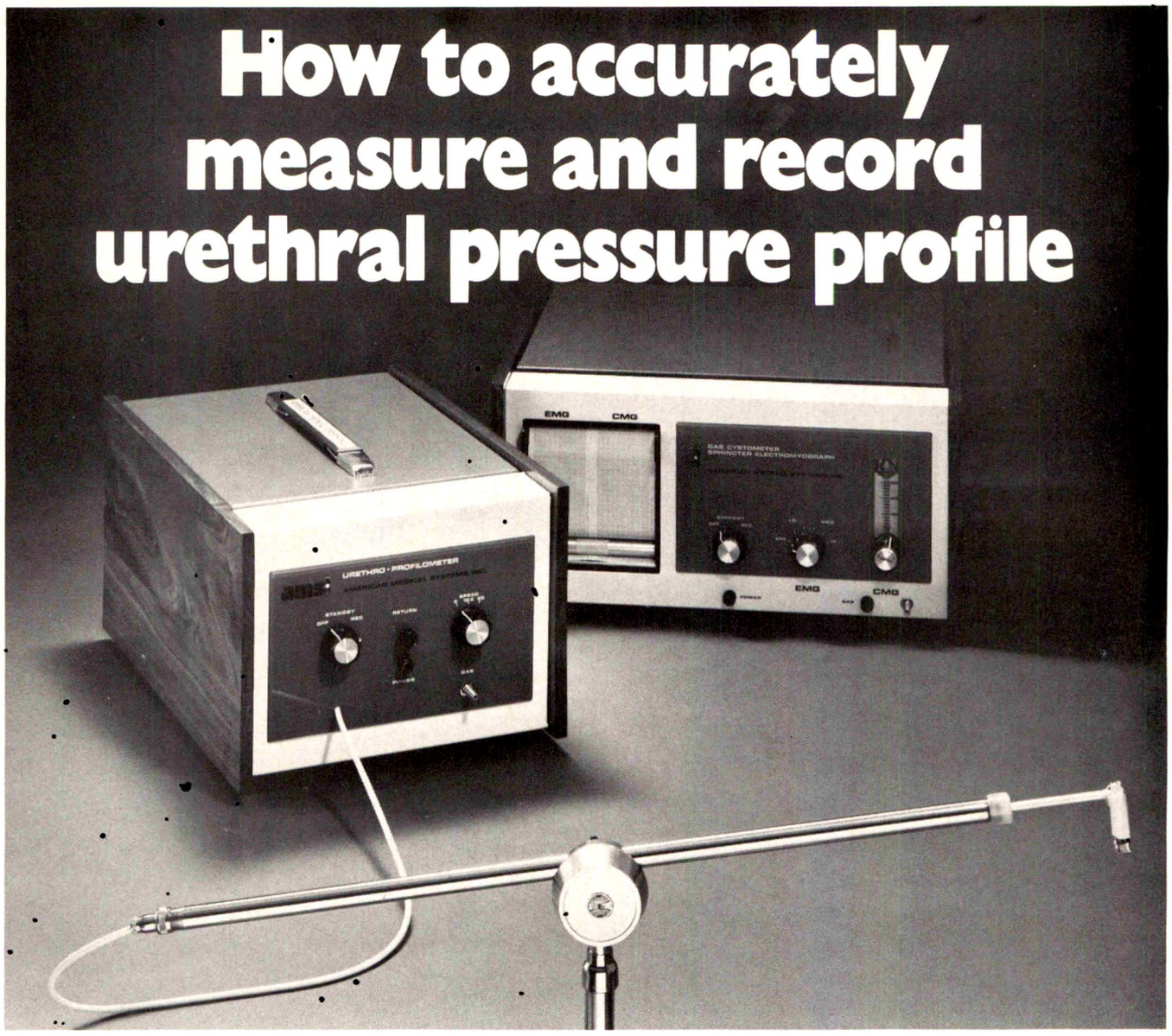
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How to accurately measure and record urethral pressure profile

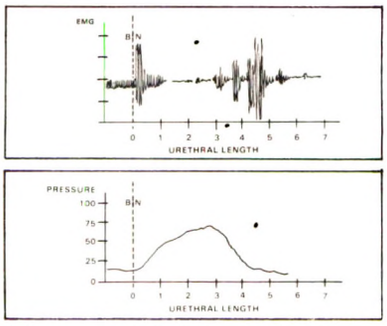


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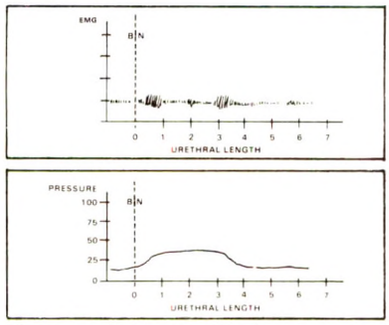
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Electromyographic and gas urethral pressure profile in a healthy female volunteer.



Electromyographic and gas urethral pressure profile in a female patient with urinary stress incontinence.



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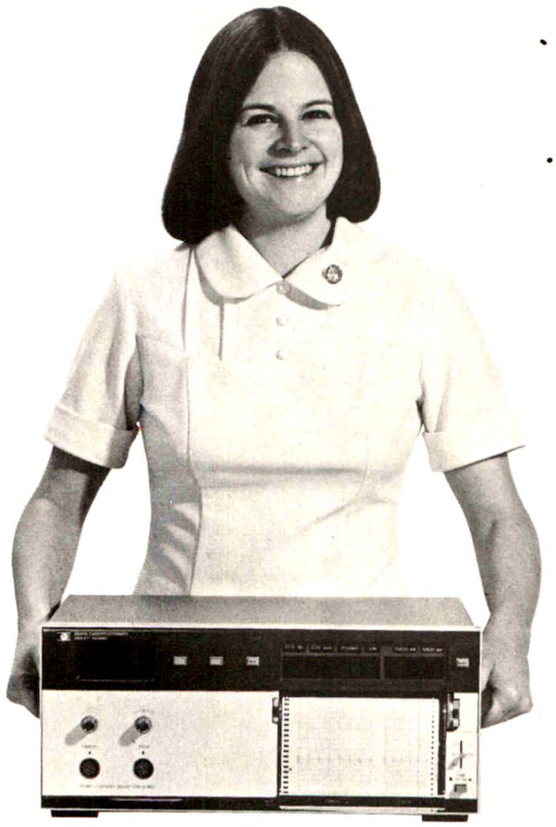
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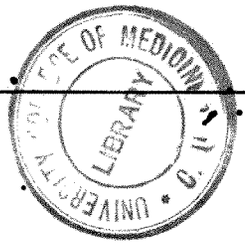
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Harold M. M. Tovell, M.D., Petra Banogan, M.D., and Artemis D. Nash, M.D.

New York, New York

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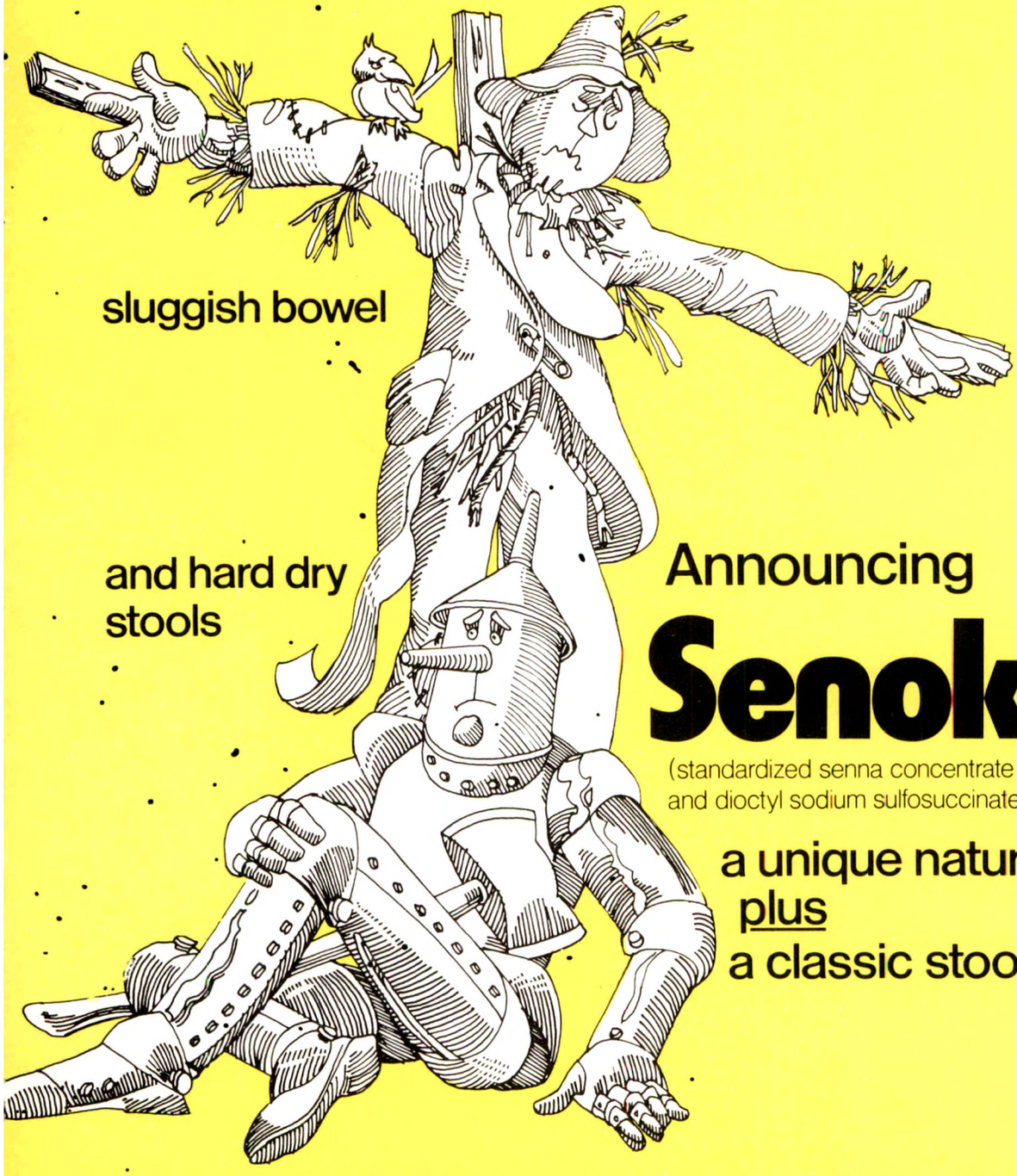
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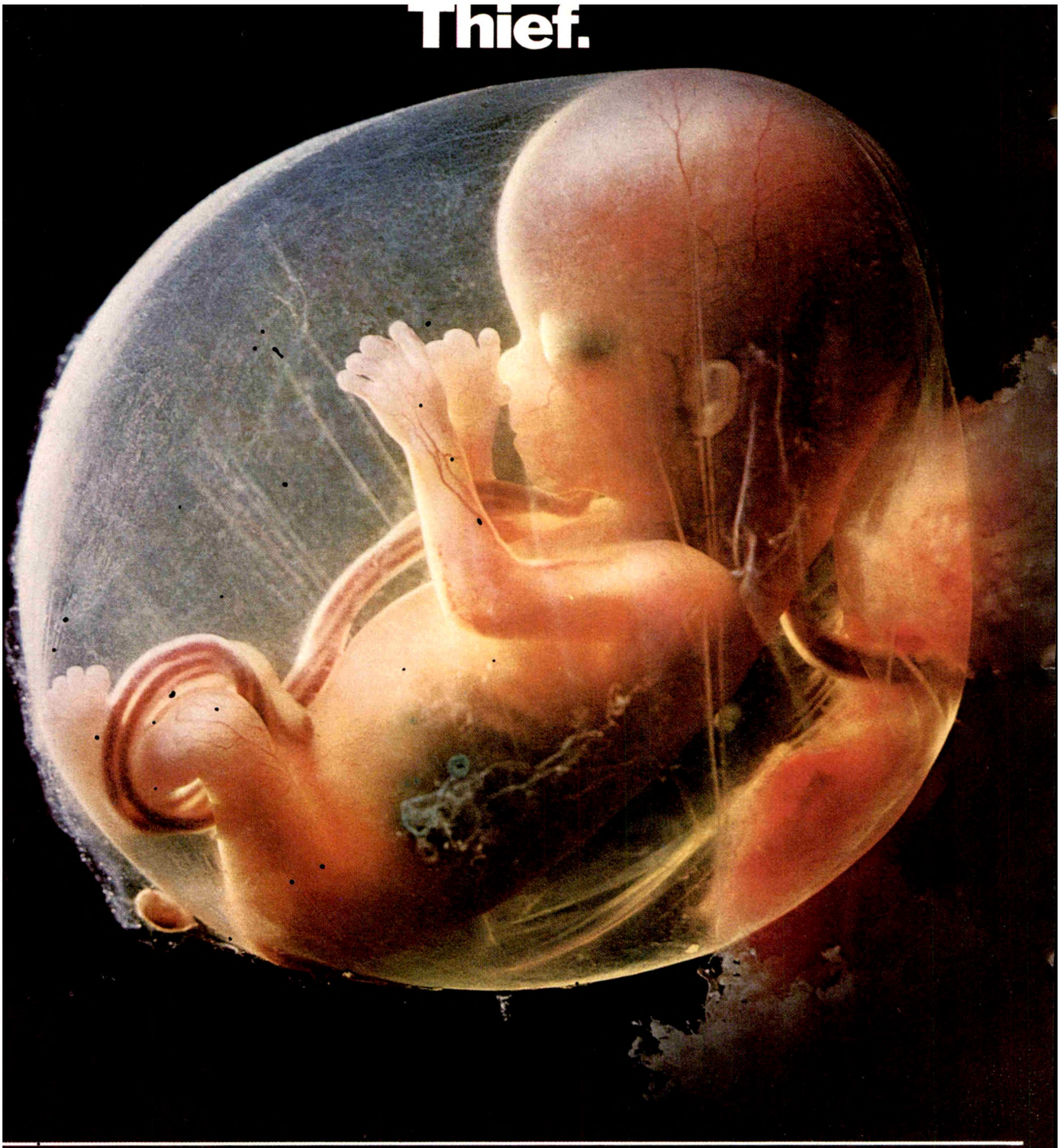
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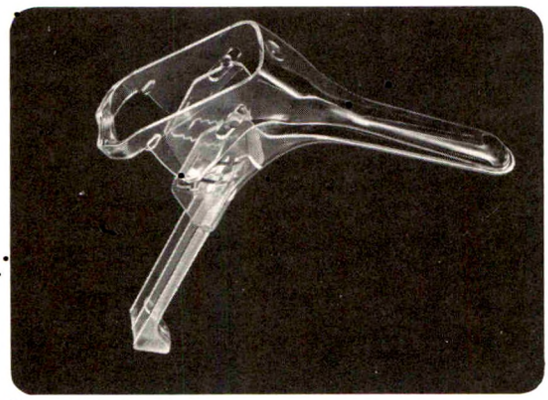
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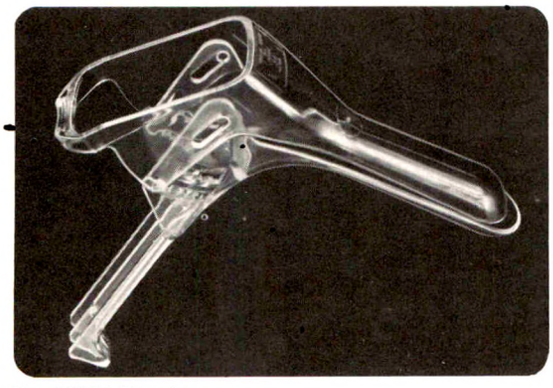
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					11,085
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IN BRIEF:

SPECIAL NOTE: Oral contraceptives have been marketed in the United States since 1960. Reported pregnancy rates vary from product to product. The effectiveness of the sequential products appears to be somewhat lower than that of the combination products. Both types provide almost completely effective contraception.

An increased risk of thrombotic, thromboembolic and cerebral-vascular disease associated with the use of hormonal contraceptives has been shown in studies conducted in both Great Britain and the United States. Other risks, such as those of elevated blood pressure and reduced tolerance to carbohydrates, have been reported, and appropriate tests should be conducted to monitor these during oral-contraceptive therapy. Liver disease has also been reported, and the physician should be alert to its earliest manifestations.

Long-term administration of both natural and synthetic estrogens in subprimate animal species in multiples of the human dose increases the frequency for some animal carcinomas. These data cannot be transposed directly to man. The possible carcinogenicity due to the estrogens can neither be affirmed nor refuted at this time. Close clinical surveillance of all women taking oral contraceptives must be continued.

INDICATIONS: For oral contraception.

CONTRAINDICATIONS: 1. Thrombophlebitis, thromboembolic disorders, cerebral-vascular disease, or a past history of these conditions; 2. Markedly impaired liver function; 3. Known or suspected carcinoma of the breast; 4. Known or suspected estrogen-dependent neoplasia; 5. Undiagnosed abnormal genital bleeding; 6. Known or suspected pregnancy.

WARNINGS: 1. The physician should be alert to the earliest manifestations of thrombotic and thromboembolic disorders, thrombophlebitis, cerebrovascular disorders including hemorrhage, pulmonary embolism and retinal thrombosis. Should any of these occur or be suspected, the drug should be discontinued immediately.

Retrospective studies of morbidity and mortality in Great Britain and studies of morbidity in the United States have shown a statistically significant association between thrombophlebitis, pulmonary embolism and cerebral-vascular disease, occlusive or hemorrhagic, and the use of oral contraceptives. There have been three principal studies in Great Britain[1-3] leading to these conclusions and three[4-7] in this country. The estimate of the relative risk of thromboembolism in the study by Vessey and Doll[3] was about sevenfold, while the United States studies found relative risks of 4.4 to 11, meaning that the users are several times as likely to undergo thromboembolic disease without evident cause as non-users.

In May, 1974, the Royal College of General Practitioners in England[8] issued an interim report of its continuing large-scale prospective study comparing a user group to a non-user group. This study in its interim analysis states: "A statistically significant higher rate of reporting of cerebrovascular accidents in Takers is evident, but the numbers are too small to justify an estimation of the degree of risk." The study also reported a higher incidence of superficial and deep vein thrombosis in users as compared to non-users.

The Sartwell study[4] indicated that the risk did not persist after discontinuation of administration. Both the Sartwell and the Royal College study indicated that the degree of risk was not associated with duration of treatment.

In a collaborative American study[5,6] of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the relative risk of thrombotic stroke was 4.1 to 9.5 times greater in users than in non-users. A comparable estimate for hemorrhagic stroke was 2.0.

None of the American studies was designed to evaluate a difference between products. However, the Sartwell study[4] suggested that there might be an increased risk of thromboembolic disease in users of sequential products.

Other retrospective studies[9,10] have reported an increased risk of post-surgery thromboembolic complications in oral-contraceptive users. It has been recommended that therapy be discontinued at least one month prior to elective surgery.

2. Discontinue oral-contraceptive medication if there is: gradual or sudden, partial or complete loss of vision; proptosis or diplopia, onset or aggravation of migraine or development of headache of a new pattern which is recurrent, persistent or severe; papilledema; or any evidence of retinal-vascular lesions. 3. Fetal abnormalities have been reported to occur in the offspring of women who have taken progestogens and/or estrogens during pregnancy.[11,12] The safety of LO/OVRAL® in pregnancy has not been demonstrated. Pregnancy should be ruled out before initiating or continuing the contraceptive regimen. Pregnancy should always be considered if withdrawal bleeding does not occur. 4. A small fraction of the hormonal agent in oral contraceptives has been identified in the milk of mothers receiving these drugs. The long-range effect to the nursing infant cannot be determined at this time. 5. Hepatic lesions (adenomas, hepatomas, hamartomas, regenerating nodules, etc.), occasionally fatal, have been reported in women on oral contraceptives. Such lesions may present as an abdominal mass or with the signs and symptoms of an acute abdomen. These lesions should be considered if the patient has abdominal pain or evidence of intra-abdominal bleeding. Such lesions have been reported in short-term as well as long-term users of oral contraceptives.

PRECAUTIONS: 1. A thorough history and physical examination should be performed before prescribing oral contraceptives and periodically during their administration and should include special reference to breasts and pelvic organs including Papanicolaou smears, since estrogens have been known to produce tumors, some of them malignant, in five species of subprimate animals. 2. Endocrine and possibly liver-function tests may be affected by treatment with LO/OVRAL®. Therefore, if such tests are abnormal in a patient taking LO/OVRAL® it is recommended that they be repeated after the drug has been withdrawn for two months. 3. Under the influence of estrogen-progestogen preparations, pre-existing uterine fibromyomata may increase in size. 4. Because these agents may cause some degree of fluid retention, conditions which might be influenced by this factor, such as epilepsy, migraine, asthma, cardiac or renal dysfunction, require careful observation. 5. In breakthrough bleeding, and in all cases of irregular bleeding per vaginam, nonfunctional causes should be borne in mind. In undiagnosed bleeding per vaginam, adequate diagnostic measures are indicated. 6. Patients with a history of psychic depression should be carefully observed and the drug discontinued if the depression recurs to a serious degree. 7. Any possible influence of prolonged LO/OVRAL® therapy on pituitary, ovarian, adrenal, hepatic or uterine function awaits further study. 8. A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. The mechanism of this decrease is obscure. For this reason, diabetic patients should be carefully observed while receiving LO/OVRAL® therapy. 9. The age of the patient constitutes no absolute limiting factor, although treatment with LO/OVRAL® may mask the onset of the climacteric. 10. The pathologist should be advised of LO/OVRAL® therapy when relevant specimens are submitted. 11. Susceptible women may experience an increase in blood pressure following administration of contraceptive steroids. 12. Cholestatic jaundice has been reported in users of oral contraceptives. If this occurs, LO/OVRAL® should be discontinued. This condition is more likely to occur in patients who have experienced cholestatic jaundice of pregnancy. Patients with a history of cholestatic jaundice of pregnancy should be carefully observed during LO/OVRAL® therapy.

ADVERSE REACTIONS OBSERVED IN PATIENTS RECEIVING ORAL CONTRACEPTIVES: A statistically significant association has been demonstrated between use of oral contraceptives and the following serious adverse reactions: thrombophlebitis, pulmonary embolism, cerebral thrombosis and hemorrhage, gall bladder disease.

Although available evidence is suggestive of an association, such a relationship has been neither confirmed nor refuted for the following serious adverse reactions: neuro-ocular lesions, e.g., retinal thrombosis, optic neuritis and hepatic lesions with or without intra-abdominal bleeding.

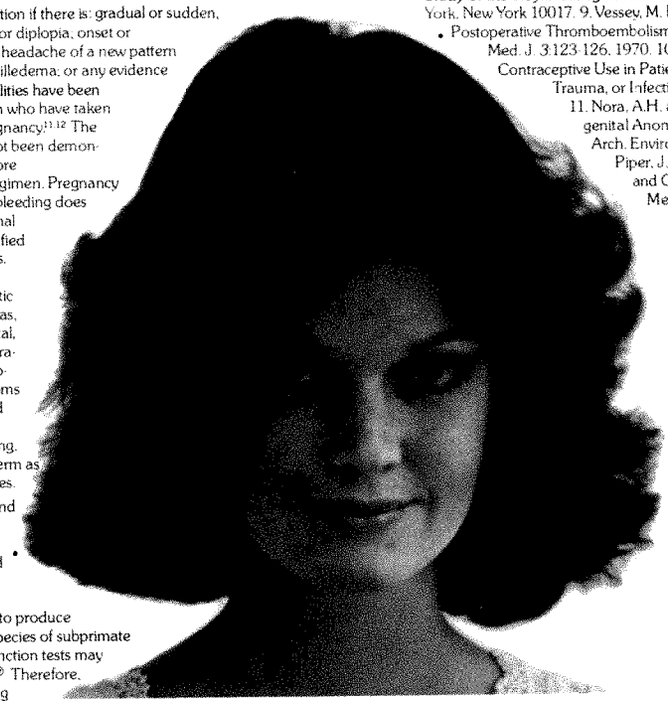
The following adverse reactions are known to occur in patients receiving oral contraceptives: nausea, vomiting, gastrointestinal symptoms (such as abdominal cramps and bloating), breakthrough bleeding, spotting, change in menstrual flow, amenorrhea during and after treatment, edema, chloasma or melasma, which may persist, breast changes: tenderness, enlargement and secretion, change in weight (increase or decrease), changes in cervical erosion and cervical secretions, suppression of lactation when given immediately post-partum, cholestatic jaundice, migraine, rash (allergic), rise in blood pressure in susceptible individuals, mental depression.

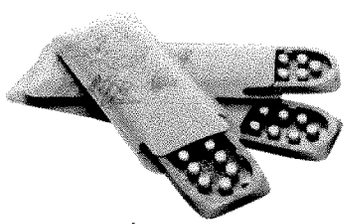
Although the following adverse reactions have been reported in users of oral contraceptives, an association has been neither confirmed nor refuted: anovulation post treatment, which tends to occur more frequently in women with a history of menstrual disorders, premenstrual-like syndrome, changes in libido, changes in appetite, cystitis-like syndrome, headache, intolerance to contact lenses, nervousness, dizziness, fatigue, backache, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, itching and vaginitis.

The following laboratory results may be altered by the use of oral contraceptives: hepatic function; increased sulfobromophthalein retention and other tests; coagulation tests; increase in prothrombin, Factors VII, VIII, IX and X; decrease in antithrombin III; increase in platelet aggregability; thyroid function: increase in PBI, and butanol extractable protein-bound iodine and decrease in T^3 uptake values; metyrapone test; and pregnanediol determination.

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The goal of learning—to be enlightened

Presidential address

CHARLES A. HUNTER, JR., M.D.

Indianapolis, Indiana

IT IS RARE that I have had the opportunity to officially extend my gratitude to an organization, such as the American Association, for offering me the substance to further a life-time goal—that of being able to influence, in a positive way, the education in our specialty. No other senior medical society has been so well endowed with all the ingredients necessary for accomplishing this goal—the goal of learning, which is the title of my address.

There are various levels of learning. Obtaining information is learning, and so is coming to understand what you did not understand before. However, there is an important difference between these two types of learning.

To be informed is to know simply that something is a fact; the acquisition of knowledge. Knowledge is a prerequisite to understanding. To understand is to be enlightened. This implies we not only know, but in addition, we perceive what it is all about. This distinction between being informed and being enlightened is similar to being able to remember something and being able to explain it.

Presented at the Eighty-sixth Annual Meeting of the American Association of Obstetricians and Gynecologists, Hot Springs, Virginia, September 4-6, 1975.

Reprint requests: Dr. Charles A. Hunter, Jr., Department of Obstetrics and Gynecology, Indiana University Medical Center, 1100 West Michigan St., Indianapolis, Indiana 46202.

Enlightenment is achieved only when, in addition to knowing what a man says, you understand what he means and why he says it. Being informed is a prerequisite to being enlightened. The point, however, is not to stop at being informed. We should strive to have as one of our lifetime goals a continuation to be enlightened.

Our continuing education depends upon books and journals and scientific meetings. Therefore, if we are to keep up to date and improve, we must know how to effectively use the learning skills. These basic skills are reading and listening.

If the physician is a poor listener or a slow reader, he is seriously handicapped. Frequently, such an individual will shun learning on a continuing basis due to boredom or because of the tremendous effort involved. We do those things we like to do. Good readers like to read.

Because the techniques of audio learning and visual learning are basic, I would like to discuss them briefly.

First, *listening*. Surveys reveal we spend almost three times as much time listening as reading. A study of various professional people reports that we spend approximately 45 per cent of our communicating time listening, 30 per cent talking, 16 per cent reading, and 9 per cent writing.

Effective listening is not easy. Real listening is a very active process. Something to challenge our intelligence. The art of listening has been said to be the art of

imaginatively moving oneself into the speaker's mind. Immediately after the average person has listened to someone talk, he remembers only about half of what he has heard, no matter how carefully he thought he listened. Two months after listening to a talk, the average person will remember only about 25 per cent of what was said. Many people who seem to be listening miss important points and, accordingly, draw incorrect conclusions from what is said. This is a serious handicap.

Effective listening—hearing, understanding and remembering—takes a great deal of willingness, effort, and concentration. Can you improve your listening? The answer is definitely "yes." Listening can be learned.

What are faulty listening habits? Some of the more common ones that have been noted are the following: (1) our minds will not wait for the spoken words—our thoughts may race ahead four to ten times faster than most people speak; (2) we are so positive we know what the speaker is going to say that we only partially listen; (3) we may miss the big idea while listening to words, and (4) our emotions and preconceived ideas unconsciously dull our ability to listen.

The other important skill necessary is *effective reading*. Effective reading implies not only rapid reading but also good comprehension. The average reading speed for medical students is in the 250 to 350 words per minute range. Associated with this reading speed, their over-all comprehension is 70 per cent. With the knowledge explosion of today, a recent Ford Foundation publication set the world's production of books at almost 1,000 a day, with scientific journals alone totaling 100,000 per year throughout the world. The accelerating pace of change is brought home by the estimate that more than half of all the research done by man has been done in the last thirty years! The knowledge explosion, in medicine and in other fields, places special demands on reading.

The words of Sir William Osler[1] were never truer than today when he wrote, "For the practitioner, a well-used library is one of the correctives of the premature senility which is so apt to overtake him. It is astonishing with how little reading a doctor can practice medicine, but it is astonishing how badly he may do it."

So much for the problem. How do you solve it? How do you make your reading more effective to keep up to date?

The first step is to become a better reader—not only to increase your reading speed but also to increase your comprehension. Reading clinics have demonstrated that the increase in reading speeds is frequently accompanied by maintaining or increasing comprehension also. To become a rapid reader is largely a matter of eye-training exercises and correcting bad reading habits. The second step to make your reading more effective is to be an analytical reader. I am not referring to reading for amusement. I am not referring to reading for factual information. I am referring to reading for understanding—enlightenment. Analytical reading is undertaken primarily for the sake of understanding. It is a demanding type of reading. Adept reading is an active mental participation, a shared endeavor, between the writer and the reader. Too frequently, readers are passive spectators.

Today, medical journals and books still constitute the principal source of medical information. The qualities we should look for in medical writings are accuracy, clarity, and simplicity. The busy and overburdened physician needs to have excellent, concise, clearly delineated written materials readily available to him. Time and relevancy are of prime consideration.

With improved reading skills and appropriate journals and books accessible, how does the busy physician have time to keep up to date? One does not *have* time; one *makes* time. Time is the most precious of treasures and the most difficult to keep. If we emulate Dr. Osler, he always found 15 minutes a day for medical reading. Now 15 minutes a day sounds like a very small segment of time. At only 250 words per minute, it means you can read approximately 26,000 words every week and over a million words each year. Since the average book contains approximately 100,000 words, this means you can read about 12 books a year. With effective practice, reading at 500 words per minute for 15 minutes a day would double the number of books per year—a total of 24 books.

To read or listen without planned goals is similar to starting a cross-country trip without a map. A clearly defined end-point or goal for each learning task should be established. Next, specific objectives should be understood by the learner. With these two anchor points, we can evolve an effective learning management plan. No matter how large or how small the topic, such an approach leaves little to chance. If the subject matter is complicated or covers a large area, it should be particularized and progress checks should be noted. At the end of the learning of a topic, appropriate evaluation should be done by the individual. If the new knowledge gained by this learning scheme not only informs you, but is understood by you, then you are enlightened.

A long-range, medical educational management program needs to be clearly defined. Starting with

undergraduate medical school, a continuum of learning should be delineated which encompasses not only the four years of undergraduate studies but includes the period of the internship and residency training.

If one of the major goals to be achieved at the end of medical school is to produce a graduate with a basic biomedical background for a lifetime of learning, then we should design our curriculum to enable him to do so. That medical education has not done a very good job in this area is apparent to most of us when we witness the stagnant effect many physicians exhibit. It is unfair to condemn the busy practitioners for not keeping up to date when we, the teachers, have not trained them in the appropriate environment during their formal training. Where have we tested and assessed the medical students and residents on their ability to listen and read effectively? Where have we really taught the student how to read a journal article critically and to evaluate the conclusions in relation to the author's hypothesis? Why are most of our evaluations relying on recall-type of testing? Why have we not clearly defined the end goals and the methods for achieving these goals for the learner at the beginning of his training?

These questions point out some of the critical areas. They are difficult questions to answer. Nevertheless, they are important and need to be answered. One of the prime goals of formal medical education is to develop in the physician the ingredients for independent learning. If this goal would be achieved, the continuing medical education of the practicing physicians would continuously upgrade medical care in all of our communities. Societal pressures and government have placed new additional demands on the medical profession. With the payment for medical services shifted from the individual patient to third-party payors and government, more and more constraints are exerted upon the physicians. New emphasis by government is expecting, and soon will be demanding, that the practitioner be evaluated periodically. The public wants assurance that competent medical services are available. Accordingly, these circumstances impose new strains on the traditional approach to continuing medical education. They call into question not so much the adequacy of the resources available, but the way those resources are used—planned, organized, managed—to meet complex educational needs. New approaches are needed—not only to the learning system but also for evaluation of medical care.

With these forces from outside the medical profession pushing for assurances that competent care is being rendered, our entire medical educational process will take on a new formality. This will be especially apparent in postgraduate and continuing medical education. An organized, common thrust will need to be developed in this lifetime of learning plan. Organized on the one hand, yet particularized in order for the learning to be responsive to each individual physician. Certainly, new directions and dimensions in this phase of our education need to be carefully planned and developed. This is urgent if we are to avoid a mockery of the forthcoming recertification.

The American Association of Obstetricians and Gynecologists has always fostered education. But, it has done much more. Through its Foundation it has financially supported and conducted learning workshops throughout the country. These workshops have proved to be valuable. Not so much for the curricular and testing materials which were developed, but more for the engendering and dissemination of a concept—learning by design, rather than by chance. These workshops were planned to "teach teachers to be teachers." One of the present programs receiving the Foundation's support is directed toward developing specific objectives for residency training. This is progressing very well.

However, a master educational plan to include not only undergraduate medical education and residency training but also continuing medical education is urgently needed. A guideline or format for a lifetime of learning in our specialty is paramount!

All too often, following formal training, we physicians tend to relinquish our intellectual drive. I ask each of you to pause in your busy life and ponder your role in developing and guiding our pursuit of learning. Will you be a part of this pursuit? Are you willing to help? I sincerely hope so.

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Immunotherapy and chemotherapy of gynecologic cancers

ROLAND A. PATTILLO, M.D.

Milwaukee, Wisconsin

Recurrent cervical, ovarian, and breast cancers have been treated with immunochemotherapy. Optimum chemotherapeutic agents have been determined by direct and indirect methods of sensitivity determination. The Hellström assay has been used for lymphocyte-tumor recognition and for cytotoxicity assays. Vaccines developed in this laboratory, consisting of autologous and allogeneic tumor-associated antigens, combined with BCG, have been used in immunotherapy. Objective tumor regression and notable clinical improvements have been observed. Forty-five advanced cases, including recurrent cancers of the cervix and ovary, along with sarcomas, breast malignancies, and melanomas, have been treated: 16 patients have died of their disease and 29 patients have survived for 6 months to 1½ years. Some of these have shown either regression, total disappearance, or stabilization of their disease process. While the results of these preliminary studies are encouraging, the as yet unknown potential of this modality cannot be expected until it is applied earlier, at a time when residual tumor cells are minimal.

RECURRENCE of a malignant tumor after full treatment by accepted means marks the point of escape from therapeutic and host control mechanisms. For this reason, the efforts of this laboratory have been devoted to attempts to understand certain aspects of reproductive tract cancer cells, their growth mechanisms, and response patterns to chemotherapeutic agents. In addition, efforts have been made to prepare tumor-specific antigens from the patient's own tumor grown in cell culture for the purpose of subsequently stimulating "host rejection" mechanisms against the specific antigens. The present study focuses upon cervical cancer with parallel studies in ovarian neoplasms, uterine sarcomas, melanomas, and breast malignancies.

Classic immunologic studies by Foley,[1] Prehn,[2] Klein and associates,[3] and others have demonstrated the presence of tumor-specific antigens in tumors of inbred mice and man.[4] Immune reactions to these tumor antigens are manifest primarily through the body's cellular immune system. This leads to the production in the host of a delayed type of hypersensitivity similar to that observed following intradermal inoculation of old tuberculin or PPD. The role of the *humoral* or *antibody*-mediated immune system in tumor immunity is not well understood; however, like organ transplant rejection, tumor rejection is believed to be mediated principally by cellular immune reactions. In fact, antibody may function as "blocking factor" and actually permit *enhancement* of tumor growth. The lymphoid cells which are active in immune reactions have been designated as "T" and "B" lymphocytes.

"T" lymphocytes are "hairy" whereas "B" lymphs are "bald," showing few microvilli on their surfaces. "T" cells are thymus dependent; "B" cells are bursa-equivalent dependent cells. They derive, in the human subject, from stem cells originally present in the fetal yolk sac and third and fourth pharyngeal pouches.

The former stem cells populate the fetal liver, the spleen, and the bone marrow before migrating to the thymus, whereupon, under thymic hormone influence, T-cell characteristics of the cellular immune system are acquired. T cells gain clinical expression by producing active *substances* called *lymphokines*. These lymphokines, designed as "factors," such as migration-inhibitory factor (MIF), blastogenesis factor (BF), transfer factor (TF), etc., serve to carry out or *amplify* the cellular immune processes. In lymph nodes, T cells

From the Department of Gynecology and Obstetrics, the Medical College of Wisconsin.

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are located in the deep cortical regions along the medullary cords and B cells are located in the germinal center of follicles.

The rationale for use of immunotherapy and for combining immunotherapy with chemotherapy

It is well recognized that skin grafts derived from genetically distinct individuals are rapidly rejected, principally by lymphoid reaction. These grafts come from foreign donors and, as their genetically distinct antigens are absorbed into the host's lymphatic system, a prompt cellular reaction is generated and subsequent rejection occurs. Malignant tumors, on the other hand, arise *within* the host and although circulating tumor cells with their tumor-specific antigens are present in the blood stream, adequate immunity and rejection do not occur. In pertinent experiments, Fisher and Fisher[5] have recently shown that the blood stream is a highly ineffective means of producing tumor sensitization. In contrast, tumor rejection could be produced only when the regional lymph nodes were intact. When the inguinal and popliteal lymph nodes were surgically excised and C_3H mouse tumors were inoculated subcutaneously in the hind leg, no immunity developed. From these two important observations, it appeared reasonable to attempt immunotherapy by stimulation of peripheral lymph nodes from their *afferent* arms. Tumor-specific antigens, when available, as well as nonspecific immunostimulants such as Bacillus Calmette-Guérin (BCG) could be used. However, before such immunostimulation could be undertaken, it would be necessary to evaluate the immunologic status of the patients to be treated. Note was taken of the fact that previous literature reports[6–13] have indicated that cancer patients have suppressed immune systems both by laboratory tests using T- and B-cell–stimulating agents and by clinical skin testing of patient responses to commonly encountered antigens. Upon documentation of a state of immunosuppression in the patients in this study, it would appear that an ample basis for immunostimulation would then have been established. Posttherapy evaluations would be expected to reflect the effects of immunostimulation by increases in lymphocyte reactivity with the test antigens or mitogens. Likewise, showing direct cell injury and cytotoxicity against the tumor cell would reflect the activity of killer lymphocytes stimulated by repeated administration of BCG and the patient's tumor antigen.

It is well recognized that ideal circumstances for potential immunotherapeutic effects are best when small numbers of residual tumor cells remain. However, because the patients in this study bear large

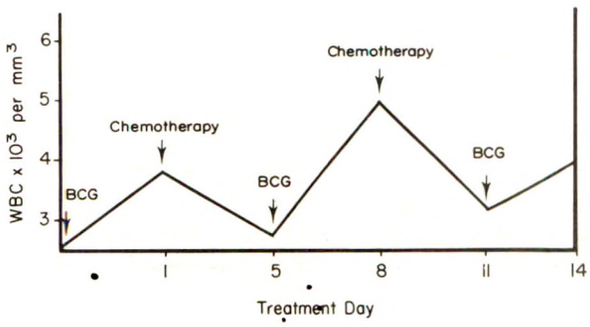


Fig. 1. Immunochemotherapy (cervical cancer)—hematologic patterns. "Bone marrow sparing" effect of immunotherapy. The "controlled infection" aspect of BCG administration produces a mild WBC elevation after prior chemotherapy suppression (Patient E. H.).

tumor loads, chemotherapeutic agents are employed to reduce tumor volume. Nonetheless, tumor cell killing with chemotherapeutic agents cannot be accomplished without inhibition of normal lymphopoietic elements and further immunosuppression. Since immunostimulation cannot be achieved in the presence of inhibiting concentrations of cancer drugs, a timed sequence must be established which will allow time for tumor cell inhibition followed by a time for administration of the immunostimulating agents—BCG and tumor antigens. This stimulation fulfills the need for inducing blastogenesis and cytotoxicity in the patient's own lymphocytes. The peak blastogenic response to BCG in vitro is approximately 72 hours and it is also noted that delayed hypersensitivity reactions to tuberculosis skin tests are roughly comparable. With these observations in mind, a schedule in which no chemotherapy is given for at least 3 days after immunotherapy would permit blastogenesis and increase in cell numbers before the next pulse of chemotherapy, which could then be administered weekly. In addition to stimulation of blastogenesis by BCG, there is also a *controlled infection* aspect of the vaccination. This effect stimulates a mild white blood cell elevation and thus provides a bone-marrow–sparing effect (Fig. 1).

Materials and methods

Immunologic methods. A total of 55 cases of recurrent malignancies of the reproductive tract have been treated and monitored immunologically. Lymphocytes were purified by Ficol gradients from heparinized blood samples obtained prior to and during therapy. The lymphocytes were incubated with plant mitogens, laboratory agents capable of eliciting blast transformation, and mitosis, similar to natural antigens. The assays were used to assess the immunologic competence of both T and B lymphocytes.

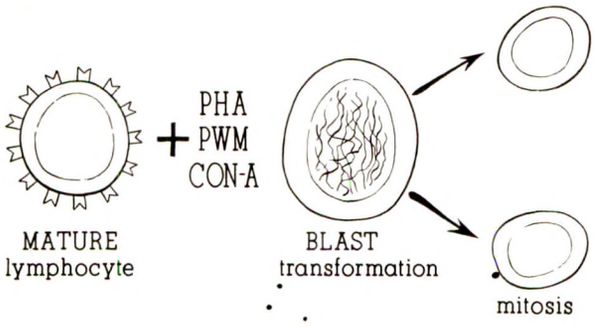


Fig. 2. Assays of afferent immunologic competence. The test measures the ability of the lymphocyte to respond to the T cell mitogens PHA (phytohemagglutinin) and Con-A (concanavalin-A) and the predominantly B-cell mitogen PWM (poke-week mitogen). The end point is c.p.m. (counts per minute) of tritiated thymidine incorporated into DNA of cells undergoing blast transformation and mitosis as compared to normal controls.

Table I. Immunologic workup

	Laboratory	*Clinical*
T cell assays	Con-A PHA	Candida antigen Mumps antigen PPD antigen SKSD antigen
B cell assays	PWM	Immunoglobulins
Cytotoxicity	Hellström assays	

Con-A and PHA are T-cell mitogens; PWM is principally a B-cell mitogen. Blast transformation and mitosis was determined by ^{3}H-thymidine uptake in DNA and recorded as counts per minute compared to normal controls (Fig. 2). T cell responses in vivo were assessed by skin tests of delayed hypersensitivity. The scheme of immunologic assessment is shown in Table I. For purposes of this manuscript only quantitative T and B cell assays are reported. Results of skin tests were similar to previous literature reports. Tumor cell recognition by sensitized lymphocytes and cytotoxicity against the tumor cells has been studied by *Hellstrom* type assay[14–16] (Figs. 3*A* and 3*B*). In this assay, tumor cells plated at 37° C. in a culture chamber are grown for 3 to 5 days to form distinct colonies. When incubated with the lymphocytes of the patient from whom the tumor is derived, or with lymphocytes from a patient with the same type of tumor, colony growth is inhibited by the individual's sensitized lymphocytes. In the presence of normal lymphocytes, no inhibition occurs. Blocking antibody can also be identified by incubation of the patient's lymphocytes in the presence of her own serum and tumor cells. In this case one observes a blockage of the inhibition of normal colony growth. In these studies for cytotoxicity, the Hellström assay has been modified to a microcytotoxicity form to measure individual cellular cytotoxicity (rather than whole colony inhibition) by its effect on inhibiting DNA synthesis in individual tumor cells (rather than whole tumor colonies).

All 45 treated patients in the study received BCG as the nonspecific immunostimulant. Ten patients with comparable recurrent cancers in the various categories received no BCG and served as control subjects.

Tumor antigen is prepared by growing the established autologous or allogenic tumor cells in chemically defined medium.[17, 18] Approximately 10^{7} tumor cells are grown in this medium free of serum, or antigenic protein for 72 hours. The medium filtrate is then concentrated to 1 mg. of Lowry protein with Sephadex G-25. This method is presently being modified to include gel filtration and ion-exchange chromatography based on preliminary studies which show specific antibody in the rabbit to an epidermoid cervical cancer antigen derived in this fashion (M. T. Story, R. A. Pattillo, unpublished data). After sterile filtration on 0.22 μ Millipore filters, 0.5 ml. of antigen was combined with BCG containing approximately 0.5 mg. of Lowry protein and spread on the acetone-cleansed skin site. Intradermal administration is achieved by pressing the disc, containing multiple tines, into the skin. The site is air or fan dried and left undisturbed for 24 hours.

Immunotherapy is initiated with nonspecific immunostimulation starting with 600 million viable BCG organisms (Pasteur Institute–Tice strain).* After laboratory and clinical evidence that immunologic stimulation has been achieved (T and B cell mitogen response and vaccination disc responses showing erythematous raised papules in vivo, and in some instances mixed lymphocyte culture response, rosettes in vitro), tumor-specific antigen from the patient's own tumor or from allogenic tumor is then added to the vaccine (Fig. 4).

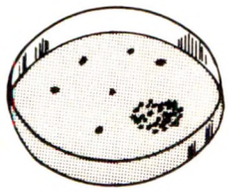
Chemotherapy methods.

Direct chemotherapy sensitivity testing. Chemotherapy sensitivity determined in vitro with the target tumor cell response to a single drug or to a battery of drugs has not been optimally utilized in the majority of previous attempts to do so. For the most part, this has been due to the limiting factor of inability to isolate pure tumor cell populations from a primary surgical specimen. As a result, much information has been obtained on the response of heterogenous cellular

*Research Foundation, University of Illinois, Chicago, Illinois.



TUMOR COLONIES



TUMOR COLONIES INHIBITED
(incubation c̄ cancer patients lymphs)



TUMOR COLONIES
(incubation c̄ normal lymphs or cancer patients serum and lymphs in Blocking Antibody Assay

Fig. 3A. Hellström assay by colony inhibition for antigenic recognition of tumor cells (assays of efferent immunologic competence). For cytotoxicity, this assay is modified to a microcytotoxicity form with inhibition of tritiated thymidine uptake into target tumor cells after incubation with cytotoxic lymphocytes compared to normal control lymphocytes. In the blocking antibody assay, the patient's lymphocytes in the presence of the patient's own serum containing "blocking factor" will block the inhibition expected from sensitized lymphocytes.

outgrowths derived from surgical excisions of primary tumors. These results have not correlated well with the response or lack of response to that particular chemotherapeutic agent in the patient's particular tumor in vivo. Unfortunately, this has led many to the conclusion that this idealistic approach to developing an in vitro tumor cell sensitivity test, similar to bacterial sensitivity tests, cannot be practically realized. Although it is unrealistic to anticipate that direct in vitro tumor sensitivity testing will be successful in every instance, a second alternative can be used. This

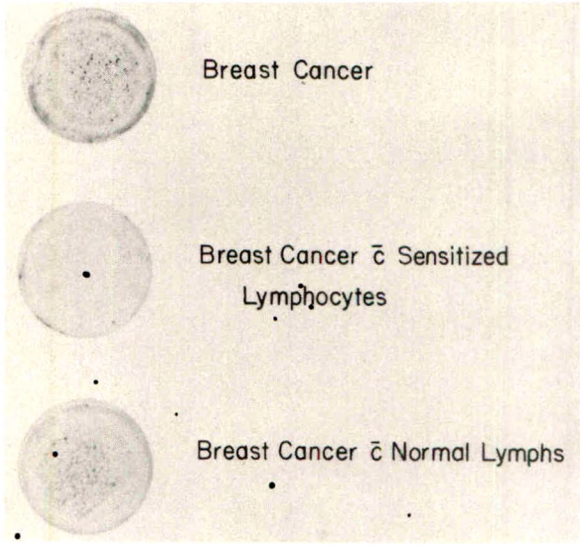


Fig. 3B. Hellström assay performed with breast cancer patients' lymphocytes. Breast cancer of established cell line incubated with lymphocytes of breast cancer patients whose sensitized lymphocytes recognized and inhibited breast cancer growth, whereas no inhibition was observed with normal control lymphocytes or in the absence of lymphocytes. Breast cancer cells were grown in Sykes-Moore chambers with lymphocytes added for assays.

alternative involves an *indirect* chemotherapy sensitivity testing method which has been used in this laboratory and clinic.

Indirect chemotherapy sensitivity testing. This method utilized model tumor systems established in this laboratory from the most commonly encountered tumors of the particular organ system. Since this effort was initiated in 1965, a series of five choriocarcinoma cell lines derived from human trophoblastic cancers have been established, as well as two epidermoid cervical cancer cell lines, a breast cancer cell line, and an ovarian cancer cell line; efforts are continuing to establish similar models from each tumor of the reproductive tract. The *indirect* chemotherapy sensitivity method requires that one demonstrate antigenic cross-reactivity of the test patient's lymphocytes in a Hellström-type assay with the target cells of the established tumor model system. Since tumor-specific cell surface antigens which characterize each tumor reflect the tumor cell's DNA, it may be predicted that the tumor cell DNA of the lymphocyte donor will respond to inhibitors of DNA in a fashion similar to that of the cross-reacting tumor model. Thus, the tumor which the patient's lymphocytes recognize from prior sensitization can be used as *indirect* chemotherapy sensitivity testing method for determining optimum drug therapy.

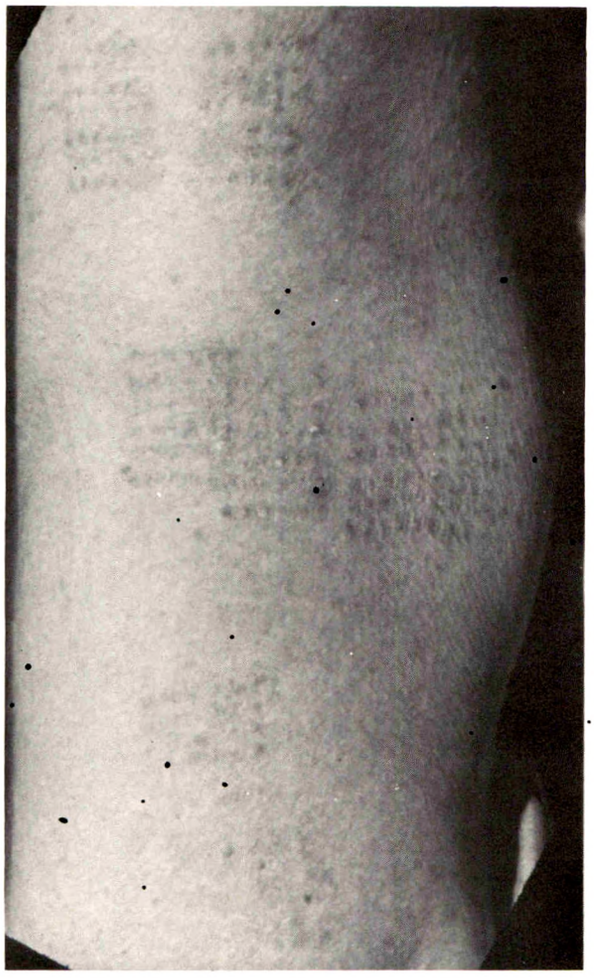


Fig. 4. BCG vaccination. The skin of the deltoid and groin areas is prepared with acetone. The Rosenthal vaccinating disc consists of 36 tines, 2 mm. in depth, on a 2 by 2 cm. base. The vaccine, consisting of viable BCG organism is applied to the skin surface and introduced into the dermis by uniformly pressing the disc into the skin with a flat magnet for pressure application. When available, tumor-specific antigen is mixed with the viable BCG organisms for specific immunostimulation. The picture shows the deltoid area of a patient's arm vaccinated in this fashion.

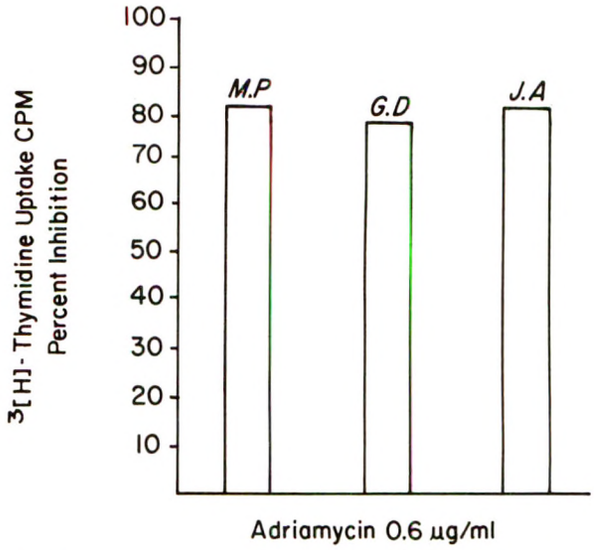


Fig. 5. Adriamycin chemotherapy sensitivity. The patient's own tumor has been grown in culture from the biopsy specimen. Cells are incubated with adriamycin (0.6 to 1.0 µg per milliliter) for 72 hours, followed by tritiated thymidine pulse for 1 hour and precipitation of DNA with 10 per cent TCA. Results are expressed as per cent of control compared to tumor cells incubated without drug. Patient M. P. (bar graph), cervical cancer; Patient G. D., ovarian cancer; Patient J. A., choriocarcinoma.

The method employed for chemotherapy incubations has been previously described.[19] Flasks (25 cm.²) containing monolayer tumor cells were incubated with the appropriate concentration of drug for 72 hours. After completion of incubation, the medium is removed and replaced by medium containing tritiated thymidine or deoxyuridine and incubated for 1 hour at 37 ° C. Following incubation, the cells are washed with cold thymidine or deoxyuridine and disrupted by freeze thawing. DNA is precipitated on a glass-fiber filter disc with trichloroacetic acid and radioactivity is counted in the Nuclear Chicago Tri Carb liquid scintillation counter. Results are expressed as per cent of control inhibition of DNA synthesis. Adriamycin incubations, for the purpose of induction of remissions, have been carried out directly with the patient's own tumor at the time of surgery or indirectly with comparable established tumor cells and the patient's lymphocytes, to identify antigenic recognition and thus cross-reactivity with the target tumor system.

Tumor recognition: Incubations of patient's lymphocytes with corresponding target tumor cells. We have used the Hellström-type assay modified to evaluate tumor cell growth after incubation with lymphocytes from cancer patients with the same histologic type of tumor. Inhibition of colony growth as originally used by Hellström as well as inhibition of thymidine incorporation into DNA, an indicator of growth, have also been used. Lymphocytes from the test patient are incubated for 24 hours with cultured tumor cells of histologic type corresponding to the patient's tumor, following which thymidine incorporation in the tumor cells is compared to tumor cells incubated with control lymphocytes. This method is used to determine previous lymphocyte sensitization to the tumor-associated antigen of the specific tumor cell. Antigenic

cross-reactivity between the test patient's lymphocytes and the tumor model, as well as quantitating cytotoxicity of the patient's lymphocytes are identified by this method.

Chemotherapy schedule. Based upon incubations of a number of the patient's own tumors tested for chemotherapy sensitivity as shown in Fig. 5, and in some instances on indirect chemotherapy sensitivity testing, all patients in the study received *adriamycin* (except the pregnant patient with melanoma). This antibiotic produces inhibition of DNA synthesis by intercalating between bases in the DNA helix, causing interruption of DNA duplication as well as inhibiting DNA transcription (Fig. 6). However, because of its toxicity and limitations, another antibiotic, actinomycin-D, which also binds to DNA and inhibits transcription, is added to the regimen before arriving at adriamycin toxicity. Because of lesser potency of the latter, two additional sites of the cell cycle can now be attacked by adding methotrexate to the regimen. Finally, in those patients responding to the first two phases, Alkeran was administered for long-term maintenance.

The immunochemotherapy schedule is shown in Table II.

Results

Forty-five patients with reproductive tract malignancies have been treated with immunochemotherapy using BCG of BCG plus tumor-specific antigens. Ten additional patients who received no BCG but received either irradiation or chemotherapy served as controls. All patients had recurrent cancers—advanced to far advanced—with the exception of one malignant melanoma which was a primary surgically excised lesion from the sacroiliac region during the last trimester of pregnancy. All patients had had full primary treatment at the time of initial diagnosis, prior to entry into the study. Included in the treated patients are 17 with advanced recurrent cervical cancers and three cervical patients treated with irradiation and/or chemotherapy alone, who did not receive BCG. Ten recurrent or persistent ovarian cancers were treated with immunochemotherapy; three received chemotherapy without BCG and served as controls in this group. Fifteen recurrent breast cancers were similarily treated, with four additional controls receiving no immunotherapy. One leiomyosarcoma and one mixed Müllerian duct tumor received immunochemotherapy; one melanoma in the third trimester of pregnancy received immunotherapy alone.

The results of treatment of 17 recurrent metastatic cervical cancers are presented. Thirteen of these had distant metastasis at the initiation of therapy: four, lung metastasis; five, bone metastasis; two, supraclavicular metastasis; two, para-aortic metastasis. 10 patients had ureteral obstruction. A similar percentage distribution of distant metastasis was also present in the non–BCG-treated control group.

The immunologic response results of combined immunostimulation and chemotherapy will be described. T and B cells were monitored by percentage increases in tritiated thymidine uptake into DNA after incubation with T and B cell mitogens. An increase in blast transformation after BCG and tumor antigen

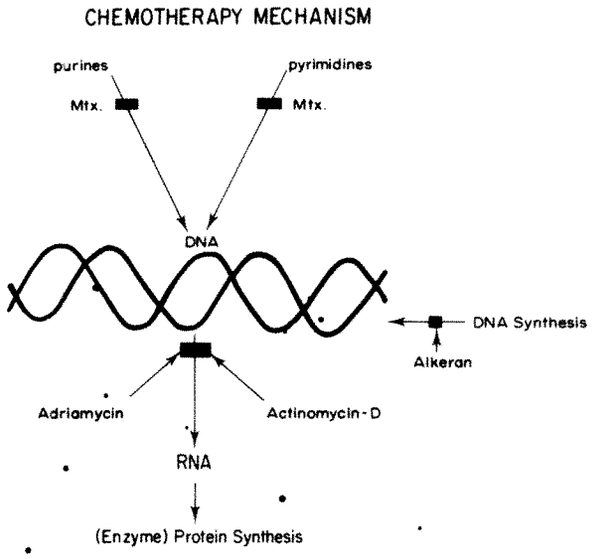


Fig. 6. Chemotherapy mechanisms. The chemotherapy schedule uses adriamycin initially for induction of remission over a 3 to 6 months period of time, followed by actinomycin D and methotrexate for the next 3 to 6 months, followed by Alkeran orally after the first year.

Table II. Immunochemotherapy schedule

Induction of remission:	
Adriamycin—0.6 mg. per kilogram intravenously weekly or 1.0 mg. per kilogram every 2 wks.	3-6 mo.
Maintenance:	
Actinomycin-D—10 μg per kilogram intravenously weekly Methotrexate—0.3 mg. per kilogram intravenously weekly	3-6 mo.
Alkeran—0.125 mg. per kilogram orally day 1, 2, 3, 8, 9, 10, after first year	
Immunostimulation:	
BCG or BCG + tumor-specific antigen weekly by disc method, day 4, 11, . . .	

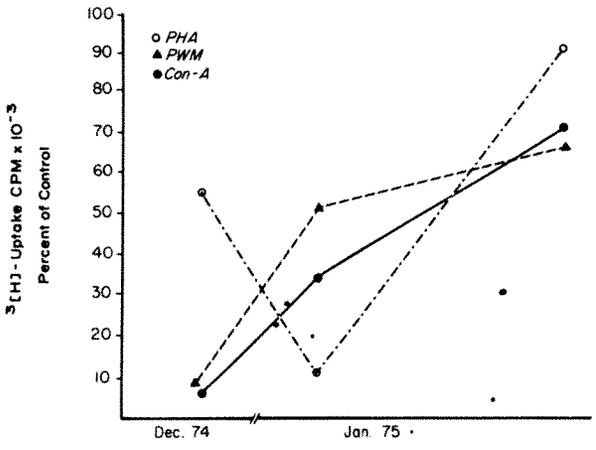


Fig. 7. Curve of T- and B-cell responses after immunotherapy (cervical cancer). Immunologic monitoring showing increased responses to T- and B-cell mitogens after treatment of patient T. D. with BCG.

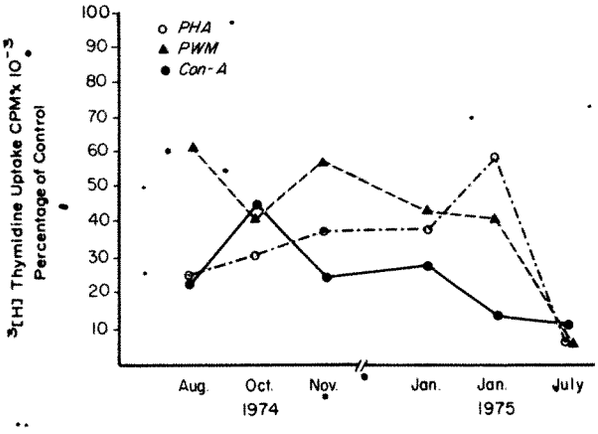


Fig. 8. Curves showing sustained levels of afferent immunologic competence in Patient E. H. with recurrent inoperable cervical cancer confirmed at laparotomy. Lymphatic obstruction and leg edema were marked at the beginning of therapy. The patient went into sustained remission and the leg edema resolved for approximately 1 year on immunochemotherapy. The sustained responses to BCG and allogenic tumor-specific antigen were maintained until July, 1975, when the biologic response to the vaccinations begin to fail and laboratory monitoring showed decreases concomitant with the beginning of a downhill clinical course in the patient.

stimulation is shown (Fig. 7), with time in a patient (T. D.) undergoing complete remission on immunochemotherapy. The patient has continued in this course for approximately 10 months. Another patient (E. H.) showed regression of palpable tumor, resolution of leg edema, and marked clinical improvement for over one year; however, she has recently begun to fail clinically and now shows failing responses to the

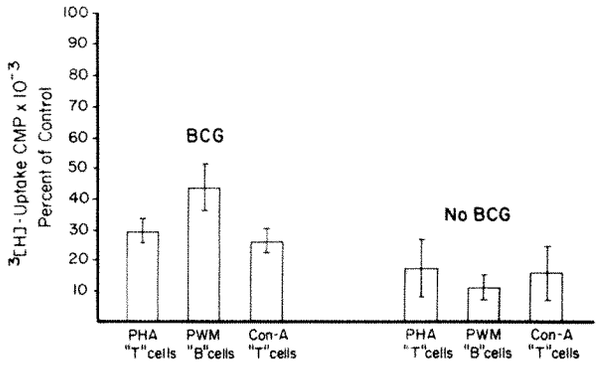


Fig. 9. Bar graphs showing the composite mean of multiple determinations of T- and B-cell afferent immunologic competence in 20 recurrent cervical cancers. Seventeen patients in the BCG-treated group all received BCG as the nonspecific immunostimulant. Three patients in the "No BCG" group received either chemotherapy or irradiation without immunotherapy. Mean responses of the BCG-treated group show increases over those of patients who did not receive BCG.

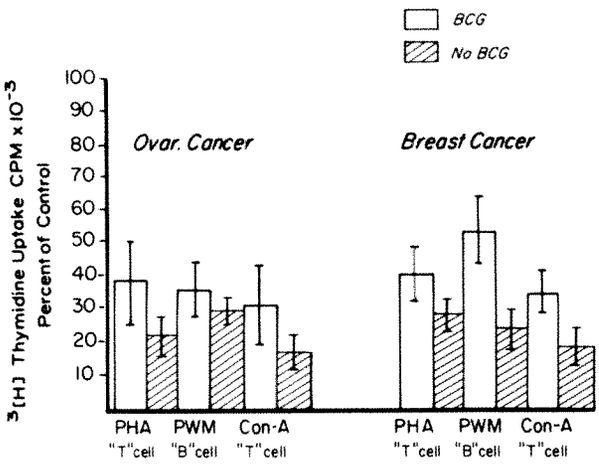


Fig. 10. Composite mean of 10 recurrent or persistent ovarian cancers treated with BCG (open bars) compared with three cases receiving chemotherapy without BCG (hatched bars), 15 recurrent breast cancers receiving BCG as the nonspecific immunostimulant (open bars), along with four cases receiving no BCG (hatched bars).

BCG vaccinations in vivo. The mitogen assays shown decline in T and B cell responses (Fig. 8).

Radiographic regression of pulmonary metastasis of epidermoid carcinoma of the cervix, documented by bronchoscopy and tissue diagnosis, was observed after immunochemotherapy in patient C. S. Regression of approximately 50 per cent of the lung lesion was noted; however, because of ileal cutaneous fistulas from mesenteric metastasis, nausea, vomiting, and electrolyte derangement, therapy could not be continued and the patient pursued a downhill course to

Table III. Clinical parameters, cervical cancer immunotherapy, 17 cases

	Pain control	*Control vaginal bleeding*	*Resolution of leg edema*	*Pulmonary lesion regression*	*Reossification of bone lesions*	*Cases responding minimum of 4 mo.*
No. of cases	7	1	2	3	1	9

*Regression interval.

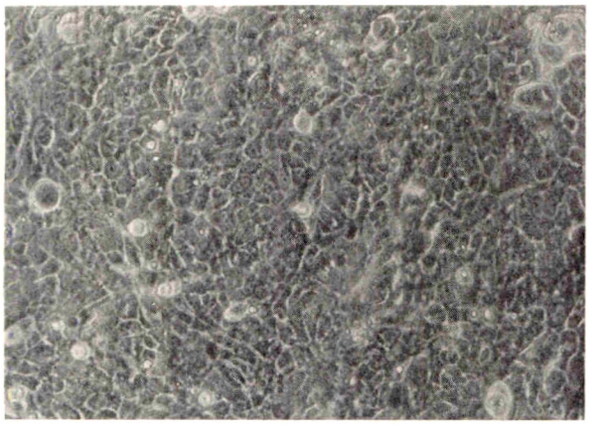


Fig. 11. Phase-contrast photomicrograph of living epidermoid cervical cancer cells (Patient T. D.) grown from biopsy of metastatic tumor invading lumbar vertebra which produced cord symptoms and required decompression. When this tumor is sectioned and stained with hematoxylin and eosin, typical epidermoid morphology, comparable to the patient's original cervical tumor, is observed. Keratinization, seen in the original tumor, is also demonstrable in the tissue culture grown tumor. The tumor cells were incubated in screening chemotherapy studies in which adriamycin was found to be most effective in inhibiting DNA synthesis. The patient received adriamycin clinically.



Fig. 12. Patient T. D.'s epidermoid cervical cancer cell is shown being attacked by the patient's own sensitized lymphocyte. Invasion of the target tumor cell by one lymphocyte has already been accomplished; a second lymphocyte is making contact at the upper pole (100×). The patient has received immunotherapy consisting of tumor antigen from her own tumor cells grown in tissue culture in Fig. 13, combined with BCG as the nonspecific immunostimulant.

die within 5 months of identification of recurrence. Symptomatic improvement in addition to radiographic regression of measurable lesions has also been noted (Table III). It was of interest that relief of pain in bony metastasis has been observed in a number of cases. This was interpreted to represent a decrease in pressure distension on bony metastasis. It was particularly noted in one patient with lytic bone lesions in the humeri and metastasis to the middle ear documented on biopsy at the time of tympanoplasty. Control of bleeding was also noted in one patient with a large exophytic cervical lesion which did not respond to irradiation or cryotherapy. Gradual resolution of bleeding over a 4 week period with reduction in size of the lesion (which had extended down to the introitus) was observed. Two patients with unilateral leg edema secondary to iliac tumor obstruction (E. H.) along with biopsied tumor in the pelvic ureter in another case (V. B.) achieved resolution of leg edema and pain. Greater than 50 per cent regression of palpable tumor was noted over a period of 1 year. Both patients have lived active lives in the presence of tumor which initially regressed and became stabilized over a period of 12 months. Most recent immunologic monitoring, however, shows decreased responses of both T and B cells in one of those patients (E. H.) corresponding now to advancing pelvic tumor.

A composite of all 20 recurrent cervical cancers is shown in Fig. 9. Seventeen recurrent cervical cancer cases were treated with chemotherapy plus BCG, and two cases with chemotherapy plus BCG, with autologous and allogenic tumor-specific antigen, respectively (Patients T. D. and E. H.). The T and B cell mean mitogen responses of BCG-treated patients are shown to be increased over those of the nontreated cases. The curves of the individual patients, as previously shown, generally correlate with tumor progression and regression. Con-A appears to be the most consistently correlated T-cell antigen, whereas PHA tends to be more variable in its correlation.

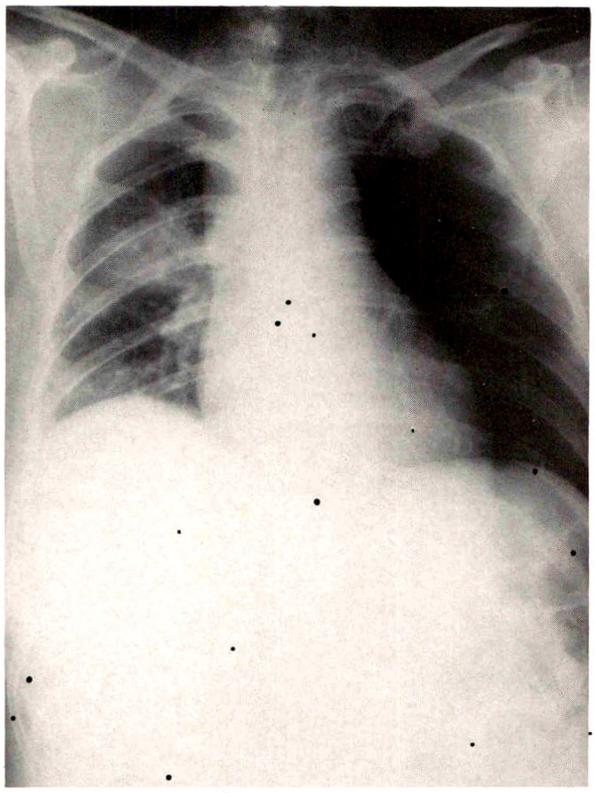


Fig. 13. Chest x-ray (Patient T. D.) showing metastatic lung lesion in November, 1974, during which time metastatic epidermoid carcinoma was identified on biopsies of the right knee joint and lumbar spine.

Table IV. Hellström assay. Patient's tumor cells ^{3}[H]-thymidine incorporation•(c.p.m. × 10^{-3})

Incubated with:	
Control lymphocytes	1.2
Patient lymphocytes	0.5

Similar results were obtained in 10 ovarian and 15 breast cancers (Fig. 10). Of the 17 patients with recurrent cervical cancers treated over the past year, seven have died, one is in complete remission, two are in partial remission, three have stable disease, and four are advancing with progressive disease. Two of the three untreated patients died within an average of 5 months from recurrence. Of 10 patients with recurrent or persistent ovarian cancers, five have died, one is presently preterminal, three have stable disease, and only one is free of identifiable tumor.

Of 15 patients with breast cancers, three have died, four are in partial remission, six have stable disease, and two are showing progression. Of two with uterine sarcomas, one died after 5 months and the second experienced regression and has remained in partial

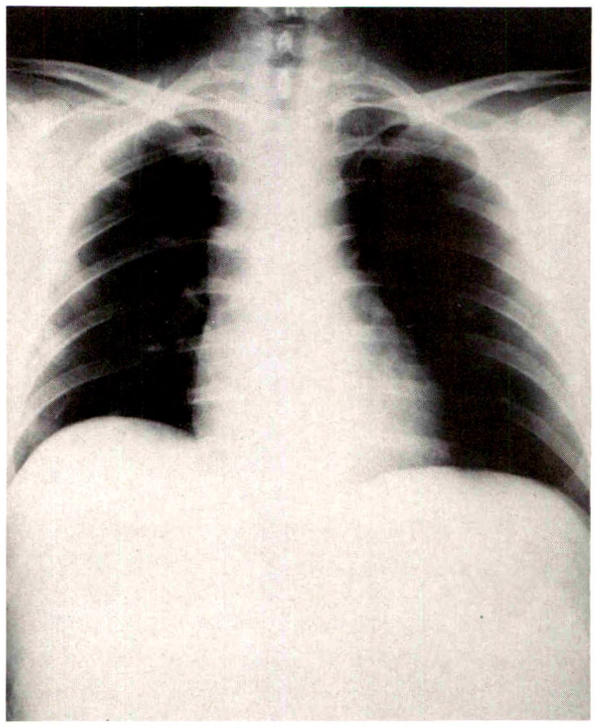


Fig. 14. Chest x-ray (Patient T. D.), March, 1975, showing resolution of metastatic chest lesion (as compared with Fig. 13) after treatment of patient with immunochemotherapy.

remission for 10 months. A patient with a single melanoma without identifiable disease after surgery was treated in the last trimester of pregnancy and has remained free of recurrent disease for 1 year.

The final case to be reported summarizes each of the methodologies used in these studies. The case is that of a 45-year-old woman with Stage I-B cervical cancer who underwent radical Wertheim hysterectomy and lymph node dissection following two intracavitary radium applications. All lymph nodes were negative for tumor. Ten months after surgery, the patient presented with cough and back and knee pain. Bronchoscopy showed a fungating metastatic lesion. Biopsy of the knee joint revealed epidermoid carcinoma and compression fraction of the third lumbar vertebra was noted. The patient underwent cord decompression and gross and microscopic epidermoid carcinoma was identified. This tumor was grown in culture from the vertebral body (Fig. 11) incubated with a screen of chemotherapy agents, of which adriamycin was most effective. The patient was treated with adriamycin and tumor-specific antigen was prepared from her tumor in culture. This was administered along with BCG by weekly vaccination.

T- and B-cell responses to mitogens after BCG stimulation of the patient showed significant increases

(Fig. 7). The Hellström assay showed inhibition of tritiated thymidine uptake into DNA when the patient's lymphocytes were incubated with her own tumor cells (Table IV). Visualization of the patient's lymphocytes incubated with her tumor showed her killer lymphocytes attacking her target tumor cells (Fig. 12). The patient's chest x-ray before therapy showed metastatic lesions in the right lung field (Fig. 13). Three months after initiation of immunochemotherapy, resolution of the chest lesion was complete (Fig. 14). No evidence of radiographic tumor can presently be demonstrated in the knee or vertebral spine. The patient is ambulatory and has returned to work. She continues to receive immunochemotherapy.

Comment

Ideally, one of the most important prerequisites for tumor immunotherapy is the presence of minimal residual tumor. However, because of the difficulty in achieving this goal when dealing with patients with recurrent cancer, and because of the inoperability of such patients, highly toxic chemotherapeutic agents are necessary. While effective in inhibiting tumor cell replication, these agents are likewise inhibitory to immune stimulatory therapy and its effect in the potential host-immune reaction. For this reason, sequential pulses of chemotherapeutic agents are withheld for at least 3 days after immunostimulation. In this schedule, peak cellular reactions to the immunostimulant are not inhibited. Lymphocytes stimulated by the viable tuberculosis organism combined with the patient's own tumor-specific antigen are caused to increase in numbers and increase their cytotoxicity against the tumor cells. Since the increase in cell numbers has already occurred when the next pulse of chemotherapy is given, these lymphocytes are left free to develop cytotoxicity.

Blocking factors, as detected in the blocking antibody assay, have been identified in a number of patients studied to date. Further results of these studies will be reported subsequently.

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Adolescent sexuality and paramarriage

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The problems of adolescent sexuality as parent and physician encounter them are briefly presented. The situation as regards venereal disease, inadvertent pregnancy, and abortion appears to be worsening. The author proposes participation of our specialty in a program directed toward (1) improved understanding between parents and child, (2) sex education throughout elementary school and high school, and (3) consideration of a new relationship between the sexes, entitled "paramarriage." Such an undertaking is a logical step in the new role of obstetricians and gynecologists as primary physicians to women.

I SHALL OFFER no apology for presenting before this organization a paper on the medical and sociological implications of adolescent sexuality, for the time is at hand when all segments of society should concern themselves with these problems. As obstetricians and gynecologists we should offer something more than contraceptive pills and surgical abortion.

While sexual experimentation and promiscuity have occurred throughout history, at no time has the degree of uninhibited relationship exceeded that which now prevails among young people.

To our branch of medicine the immediate concerns are the increase in illegitimate pregnancy[1] and the spread of veneral disease.[2, 3]

To parents, the problem is the loss of contact with adolescent offspring and the difficulty of maintaining moral standards in the home.[4] Sexual freedom has advanced to the point that parents wonder whether they are sending their children to college to acquire an education or to participate in a vast experiment in free love.[5]

Psychologists are concerned with the emotional problems of the girl who, though preferring to relate her sexual experience to marriage and childbearing, now, with old inhibitions removed, exposes herself prematurely to illicit relationship, possible pregnancy, and oftentimes abortion.[6]

Churches acknowledge their impotence in the face of changing mores and waning standards.

Finally, young people themselves are disturbed about their own independence of action, the effect it may have upon their position in the community, the alienation from their parents, and the loss of guidance which may occur in the future.[7]

Into this area of confusion the issue of liberal abortion has been injected by the action of the United States Supreme Court on July 22, 1973.[8] The response to this action has been varied and vigorous.[9]

For instance, ecologists indicate with satisfaction a fall in birth rate. Public health officers note a reduction in the morbidity and mortality rates of clandestine abortion. The sexually free have acquired a legitimized form of postconceptional contraception.

On the other hand, the Catholic Church views legalized abortion as little short of legalized murder. Other segments of society find it difficult to accept an arbitrary week in pregnancy as the date when a conceptus becomes untouchable human life, and view the court action as ill advised. Several states have voiced opposition and it is quite possible that the action of the Supreme Court may ultimately be revised by constitutional amendment.

It is now noted with some concern that the number and proportion of legal abortions among young people is increasing, as if the operation itself solicits among them a growing clientele.

Obstetric clinics are finding difficulty in adjusting to the new situation. They find themselves in the anamalous position of devoting half their time to the *saving* of fetal life in late pregnancy and the other half to *destroying* it in early pregnancy. The result is an assault upon our much-vaunted ideals of pregnancy salvage.

Reports from countries which have had long experience with free abortion indicate a low immediate morbidity rate but a rise of complication rate in

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subsequent pregnancy. They express the hope that the operation can soon be replaced by adequate and acceptable methods of contraception.[11]

There exists an extensive literature on these several subjects. From it I will quote several observations which seem generally accepted and pertinent.[12]

1. Fifty per cent of adolescents between the ages of 15 and 19 engage in sexual intercourse. By the senior year in many colleges 70 per cent of the single students of both sexes engage in intercourse.

2. Of those who are active sexually, half do not use contraception. Practically none use contraception on their first sexual contact.

3. Ten to 15 per cent of sexually active adolescents have had venereal disease.

4. Most adolescents consider sexuality a natural phenomenon and not immoral. There is a growing tendency among them to pursue a monogamous relationship in which they find not only biologic gratification but also a meaningful situation in which sexuality is only a part.

5. As regards marriage, 90 per cent say that some day they will probably want to marry and have children, but the majority feel unprepared to assume the financial and other responsibilities at their time of life.

6. With it all, young people feel insecure about their sexual attitudes and behavior, and would like their sexuality to be recognized. They feel guilty about concealment and troubled that they have no common ground on which to discuss their problems with their parents.

7. Those who are sexually active are concerned about pregnancy, abortion, and venereal disease and wish that more information had been given them at various stages of their schooling.

These are the facts regarding one of the troubled areas of our times. Like many other problems, this one presses us for solution. What can we do about it?

Early training

All of us, I believe, would like our children to be understanding of sexuality and its place in life, affectionate toward fellow beings, capable of marriage, prepared to establish a home, and competent to rear their own offspring. Such a goal requires planning and effort and in most instances an early start with the persons concerned.

Psychologists tell us—and, in this case, I think correctly—that the newborn infant's mind is an open slate upon which early impressions are written indelibly. The first impression perhaps is the reception into this world, which may be warm and affectionate or cold and rejective. Experience in our delivery service leads me to believe that natural childbirth, optional presence of husband, early contact with mother, immediate and sustained breast feeding, and rooming in all contribute to affectionate family relationship. In normally progressing births I believe these policies should be encouraged.

Warmth of relationship should be continued in the home to the end that the child will not hesitate to discuss its problems with its parents. In these respects the obstetrician and gynecologists should set the example, for to what other authority are the parents more likely to look for guidance?

It is hoped that as regards a deep sex relationship that the daughter may learn to say "No" until she is fully prepared to say "Yes." In this connection it is wise to point out that virginity is not an unhealthy state, that sex relations are not essential to a full and happy life, and that many young men still prefer to marry a virgin in preference particularly to a young woman who has participated in multiple illicit relationships. Also that there is nothing thus far to indicate that marriage is less successful because husband and wife experience their first sexual intercourse together.

Sex education in the schools

There is wide difference of opinion regarding the place of sex education in the schools, stemming from the questions of when to introduce the subject, what to teach, and by whom the teaching should be done. Authorities now recommend that sex education be started in the first year in a simple and limited fashion, and expanded in the grades of elementary school and high school. Ultimately the entire subject, including the control of pregnancy and the avoidance of veneral disease, would be covered. A child so enlightened could view sex as a natural phenomenon, freed from the furtiveness, concealment, and half-knowledge that now surround the subject.

Books which treat graphically and thoroughly of sex in this fashion are now available to teach teachers how to teach school children.[13, 14] These could be reviewed by our profession and recommendations made to enlist the support of parents to such a program. The day is long since past, however, when one lecture given by one physician to one class in high school can be expected to fulfill the needs of the situation.

Fortunately or unfortunately, as one views the problem biologically or esthetically, neither family ties, nor religious fervor, nor education, nor the promise of ultimate marriage serve to supress the sexual drive of adolescent youth. Modern society provides no accepted direction for the release of sexual energy other than

formal marriage. As a result there has come to exist the current "living together" of mature persons[15] and the parasexual activities and illicit sexual intercourse of young people under a vast canopy of curiosity and ignorance.

Attempts have been made through history and in several civilizations to bridge the years between sexual maturity and the time of marriage. Primitive peoples grant sexual privileges and perform tribal rites at puberty. Based upon experience in the divorce courts of Denver, Colorado, Judge Ben B. Lindsey proposed in 1926 a form of companionate marriage.[16] In 1966 Margaret Mead[17] suggested two-stage marriage, the first stage permitting cohabitation without insoluble commitments and the second stage involving formal contracts, establishment of the home, and the rearing of children. Neither of these proposals "caught on," either because the time was not right or the terms were not suitable.

Now I believe the time is at hand for reconsideration of such proposals and the establishment of a new and additional relationship between the sexes. I have chosen to call this "paramarriage."

Paramarriage

Paramarriage could be defined as a legalized relationship permitting cohabitation between a healthy male of 18 and female of 17. No economic support or common taxation would be involved. No change of name of the female would be necessary.

In case of issue the offspring would be legitimate and registered as such. It would bear the family name of the father. The parents could marry but in any case they would be held mutually responsible for the rearing of the child until of age.

Application for Paramarital Certificate would be made by both parties one month before the date of paramarriage. In the interval they would be subject to physical examination, blood test for syphilis, and culture for gonorrhea by a physician. Contraceptive measures would be made available.

Paramarriage could be annulled after two years upon written notice submitted four months in advance by either partner. Notice of such annulment would also be given to the legal authorities.

Paramarriage could progress into formal marriage by simple adjustment of license.

Comment

By the approaches outlined above several of the problems of adolescence could be resolved.

The program of graded education in the schools would give the subject of "sex" a much-needed airing. Cultivation of a cordial home environment and improvement in child rearing would encourage the adolescent to discuss problems freely. Paramarriage would provide companionship, a legitimate area of intersexuality, and preparation for formal marriage. Young people could also spend the years necessary in preparing for their careers. By early contact with young persons physicians could reduce the spread of venereal disease and reduce the occurrence rate of abortion.

However, any approach to the problems of adolescent sexuality will require compromise on the part of a number of individuals. The parents will have to devote more time to their children. If children are given additional privileges they must display greater responsibility. If liberal abortion is to be done away with, contraception must be accepted as a universal policy. If obstetricians and gynecologists are to fulfill their role as primary physicians to women they must demonstrate greater leadership.

Though the effort be considerable, the ultimate result could be rewarding.

Thanks are due to Alice Mackov, Reference Librarian of the Samuel Scott Memorial Library of Thomas Jefferson University, and her staff, for help in locating and securing items of reference.

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Discussion

DR. CLAYTON T. BEECHAM, Danville, Pennsylvania. It is a privilege to discuss this paper and in so doing honor a Fellow who has contributed to the Association since 1930 and to our specialty even longer. Dr. Montgomery's stewardship to obstetrics and gynecology has refreshed and nourished all of us privileged to work and associate with him. Today, he is calling upon us to accept leadership in a newly acknowledged gynecologic area—sexuality.

Wisely, Dr. Montgomery has not told us precisely what should be done but he asks us as obstetricians and gynecologists to accept responsibility—accept the fact that today human sexuality exists "under a vast canopy of curiosity and ignorance." With this acceptance comes recognition of sexual behavior as a major problem which affects both physical and mental health. That brings us directly into clinical medicine—more specifically, psychosomatic or psychosexual gynecology.

To many practicing gynecologists there is something vaguely unacceptable about the whole idea and to most of us our professional absorption with clinical and surgical orientation leaves little room for interest in this area.

Instructional Objectives for a Basic Curriculum in Obstetrics and Gynecology, ed. 2, published by the Association's Foundation, has been received by most in this audience during the past two weeks. If the senior members of the Association have had time to review this work they will note extraordinary changes since their medical school days. In keeping with the subject Dr. Montgomery has introduced, the *Basic Curriculum* gives as much editorial space to sexuality as to endocrinology and much more than it does to neoplasia and other disabling physical conditions. The unit on Human Sexuality is introduced with these words: "Women who have questions or concerns regarding sex have few dependable people to whom they can turn for advice. The person who can best give such advice must understand her concern or problem, her particular medical condition, and human sexuality in general. In preparing to become a physician the student must therefore have a thorough knowledge of sexual mores, myths, practices, physiology, and dysfunction. In order to educate and treat the patient who has concerns about sex; the student must develop skills in communication and counseling." These are appropriate charges for today's clinician. However, what follows in the *terminal objectives* (for the student) is deficient when it does not pull together the relationship of sexuality to psychosomatic complaints.

As an example, let us consider briefly an actual case of a wife-mother who seeks gynecologic consultation for the ubiquitous complaint—lower abdominal discomfort. Neither her history nor a physical examination is helpful. As the visit draws to a close she blurts out a question: "Is it harmful to swallow my husband's semen?" You quickly answer, "No." The next time she comes for a checkup she relates that her pain disappeared soon after her last visit.

The number of women who visit the obstetrician-gynecologist with physical complaints which are basically emotional response to their guilt, anxiety, and/or dissatisfaction with their sex life is legion. Often their complaints defy description, or may be so ordinary as to lead the gynecologist into reaching for his laparoscope. However, it is plain that sexual aberrancy as well as sexual dissatisfaction frequently ends up in the gynecologist's office asking for help. It is not easy to determine this in taking the conventional history.

What are we going to call this Minotaur which, we realize, is an ancient subject coming under clinical scrutiny? Is it behaviorism, psyche, sexuality, or psychosexualtiy?

The semantics are important so that we talk about and treat (if possible) the same entity. I prefer psychosexuality and hold no brief for those who wish to include its recognition and treatment as anything more than clinical art. It is definitely not a science.

I believe that psychosexual gynecology is as much a part of our specialty as oncology or high-risk pregnancy. And while we accept that responsibility let us recognize that, for the first time in human history, sexuality to a woman carries no fear of an unwanted pregnancy.

For those in our specialty who develop skills in handling psychosexual problems I extend my admiration and respect, but I do ask that you be charitable to those of us who find the operating room and the obstetric unit our happiest clinical milieu.

DR. JOHN J. SCIARRA, Chicago, Illinois. Dr. Montgomery is to be commended for drawing our attention to a topic that without question is worthy of consideration by our specialty. It is inescapably apparent that adolescent sexuality is a reality and its sequeleae often have important physical, emotional, and social consequences.

The negative effects of teenage sexual activity can often be seen in unwanted and unplanned pregnancy, abortion, venereal disease, and emotional illness. We, as obstetricians and gynecologists, are immediately concerned with these problems. It would be socially irresponsible not to agree with Dr. Montgomery that our assistance should involve more than the provision of contraception and surgical abortion. Young people need correct and factual information and, too often, efforts to provide it by both educators and physicians have been ineffective.[1] Friends are still the teenager's major source of sex information. Many young people seek birth control assistance but find it difficult to acquire.[2]

Sexual activity among teenagers may not be as casual or as uninhibited as Dr. Montgomery suggests. Certainly, several recent studies of adolescent sexual behavior and attitudes, such as the one published in 1973 by social psychologist Robert C. Sorensen,[3] or the extensive national survey of sexual behavior in the 1970's reported by Morton Hunt,[1] indicate that the majority of young people cannot be properly described as sexually promiscuous, uninhibited, or irresponsible. Although some teenagers stress the purely physical aspects of sex, far more speak of the special meaning it has in a caring relationship, and in expressing or engendering loving feelings. Adolescent sexuality is closely bound up with human values. Teenagers talk about sex, not as being inherently right or wrong, but in terms of the purpose for which it is used. Premarital intercourse is considered acceptable where there is strong affection. Sorensen's group found among many of the adolescents they questioned an interesting tolerance of predominantly adult attitudes or behavior that they would definitely reject for themselves. In questioning 2,026 American adolescents and adults, Morton Hunt and his group found that, while casual sex does exist, it occurs chiefly among somewhat older single, separated, and divorced persons; it is not typical of adolescents. Dr. Sadja Goldsmith and her associates,[4] interviewing 377 teenage girls attending California Planned Parenthood Teen Centers and maternity homes, found that the reasons for having intercourse given by a substantial majority of the girls involved mutuality of desire or a mutual wish for love and closeness. These attitudes hardly constitute a disturbing departure from the cultural values held by many responsible adults.

In discussing a topic such as adolescent sexuality, about which people have strong feelings and opinions, the terminology used frequently reveals one's underlying attitudes. I think it is important for us to make an effort to avoid words that are judgmental or have negative behavioral connotations. We should be wary of such highly connotative terms and phrases as illicit relationships, illegitimate pregnancy, and free love. I believe that it serves no purpose to speak of abortion as an assault on our ideals of pregnancy salvage. Similarly, we should avoid dramatic statements about society's inability to suppress the sexual drive of adolescent youth.

I agree with Dr. Montgomery regarding the psychological benefits of family-centered maternity care and the need for early and honest sex education. I would like to think that the time will come when young people will be so well prepared for their role as sexual persons that their adolescent years will not be marred by the problems of unwanted pregnancy or venereal disease.

I doubt that any reasonable person would disagree with Dr. Montgomery that adequate methods of contraception are preferable to abortion, but the idea that the increasing number of abortions among young people can be attributed to the possibility that "the operation itself solicits. . . a growing clientele" is questionable. A common argument used by persons opposed to providing contraception and abortion services to young people is that availability *per se* leads to increased sexual activity. Studies show that this is no more true than that the denial of access to these services succeeds in discouraging sexual activity.[5]

Any proposals that we offer to deal with the problems engendered by adolescent sexuality must be acceptable to young people themselves in order for these proposals to work. One major area of difficulty between the generations is that teenagers seem not to be looking to adults for answers; they want to decide for themselves what is acceptable sexual behavior. Teenagers tend to be critical of advice based on their parents' recollections of their own adolescent sexuality, feeling that adults do not listen to, or take seriously enough, the ideas and opinions of teenagers, with the result that they are largely ignorant of what sexuality is like for teenagers and what their values area. Sorensen found that adolescents have less respect for their parents' opinions about sex (65 per cent) than about their opinions in general (80 per cent), and concluded that, "The majority of young people believe that society's rules about sex are for society's own benefit rather than their own. Young people do not deny their membership in society, but their generational chauvinism often causes them to speak of society as though it were an organization or force apart from themselves."

The area in which parental and adolescent values come into the greatest conflict seems to be the importance of marriage, and the related matter of premarital intercourse. Sorensen's group found that 76 per cent of all boys studied and 66 per cent of all

girls agree that two people should not have to get married just because they want to live together; young people are considerably less tolerant of extramarital sex than they are of sex without marriage. The main conclusion Hunt's group drew from their data was that the increase in premarital intercourse in recent years indicates an unwillingness of young people to accept what is to them an unrealistic and unnatural prohibition of all premarital intercourse. Seventy-three per cent of Sorenson's boys and 60 per cent of the girls believed that marriage is just another legal technicality for two people who love each other and are living together.

A concern I have with the paramarriage concept is that it might tend to tie young people into an exclusive and legal relationship at a time when they much need to explore a variety of relationships. Another concern is that, on the basis of what we have learned about the priorities and values of adolescents, they might reject paramarriage as a mechanistic and irrelevant device geared mainly to the needs of parents who cannot accept the idea of sexual intercourse outside the framework of marriage.

In defense of paramarriage, I will say that it is designed to come to terms with the problems of adolescent pregnancy and the spread of venereal disease. Should paramarriage or a similar form of companionate or two-stage marriage be accepted, it would insure that sexually active teenagers would receive adequate health care, it would provide an opportunity for contraception counseling, and it would provide a means of protecting and assuring the care of babies born to young couples.

Dr. Montgomery has presented a very interesting and thought-provoking essay and has fulfilled his intention "to arouse the interest of the profession and to enlist the help of our colleagues in finding a possible solution, rather than providing a perfect and final remedy."

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DR. FREDERICK J. HOFMEISTER, Wauwatosa, Wisconsin. I hesitated to rise and discuss this paper, but as a clinician I thought an observation which I have made over the years is important. So frequently the most sophisticated young lady, often a high school or college student, needs help. After the original interview and at times after a subsequent interview it is clear that there had been a lack of clarification of understanding regardless of what degree she holds, what school she has gone to, and her sexual contacts with a male.

The educational aspects of lectures, the sex lectures, the premarital courses, or whatever may be given—and I participate and have for years in so-called premarital counseling—still do not always offer the clarification and reassurance that the clinician can if he is an interested and concerned physician. The history taken before the examination does not always disclose the questions of this concerned girl; however, to the physician the examination may reveal secrets and expose the fears of the young lady. Once revealed, it is your responsibility to, without offense, and while examining, ask the patient questions rather than expect her to ask you questions. It is not uncommon to say, "Are you doing anything to protect yourself during your sexual contacts?: She often quickly answers, "Oh, no." "You are having sexual contacts?" She says, "Yes, but only occasionally." It is your job to then clarify the necessity for protection and stress that it takes only one contact. Encourage her to ask questions. Thus, confidence will be established and you, as her physician and hopefully as her future counselor, will be asked to continue to provide education in family life and family living.

DR. KEITH P. RUSSELL, Los Angeles, California. I rise to respond briefly to Dr. Montgomery's somewhat dire presentation. I have great respect for Dr. Montgomery and his views; however, I believe that the picture which has been painted in a rather negative manner is not all bad. Let me point out just one small area in which it is easy to highlight all the bad things, and that has to do with the development of legal abortion.

No one will deny that evils have been carried out in the name of legal abortion, and we still have the venal and economically oriented operators in our midst. However, as a result of a responsible approach to abortion, there has been developed a type of medical-social counselor qualified to deal with all of the aspects related to Dr. Montgomery's thesis. By use of such personnel, the utilization of abortion has been reduced in many situations and a responsible use of contraception has been advanced. This has been one of the more beneficial developments of our changing times, and such counseling has very often led to exactly the outcome which Dr. Montgomery has proposed and given the title of "paramarriage."

DR. MONTGOMERY (Closing). Dr. Beecham has properly drawn attention to the fact that physicians themselves need instruction in the problems of sex, and that this instruction should be incorporated in the

undergraduate curriculum of obstetrics and gynecology.

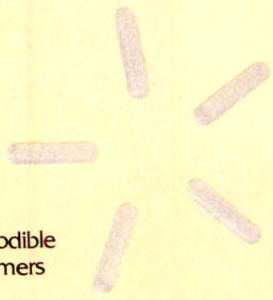
Dr. Sciarra doubts that adolescent promiscuity is as widespread as present-day observers would have us believe. It is true that dependable statistics on this subject are hard to come upon; however, for one who has followed this problem for a number of years, it seems obvious that it is an issue of growing proportions and increasing significance. I would agree with him that the majority of adolescent relationships are of a monogamous character and would hope that paramarriage would support and strengthen this tendency.

The purpose of this paper was not to criticize the adolescent but to recognize his many social problems and to help him find a solution to at least one of them. Perhaps if his sexuality can be properly provided for, the other issues of alcoholism, narcotic addiction, violence, and vandalism may become less pressing.

Advanced course in colposcopy with video tape tutorial, July 22-24, 1976

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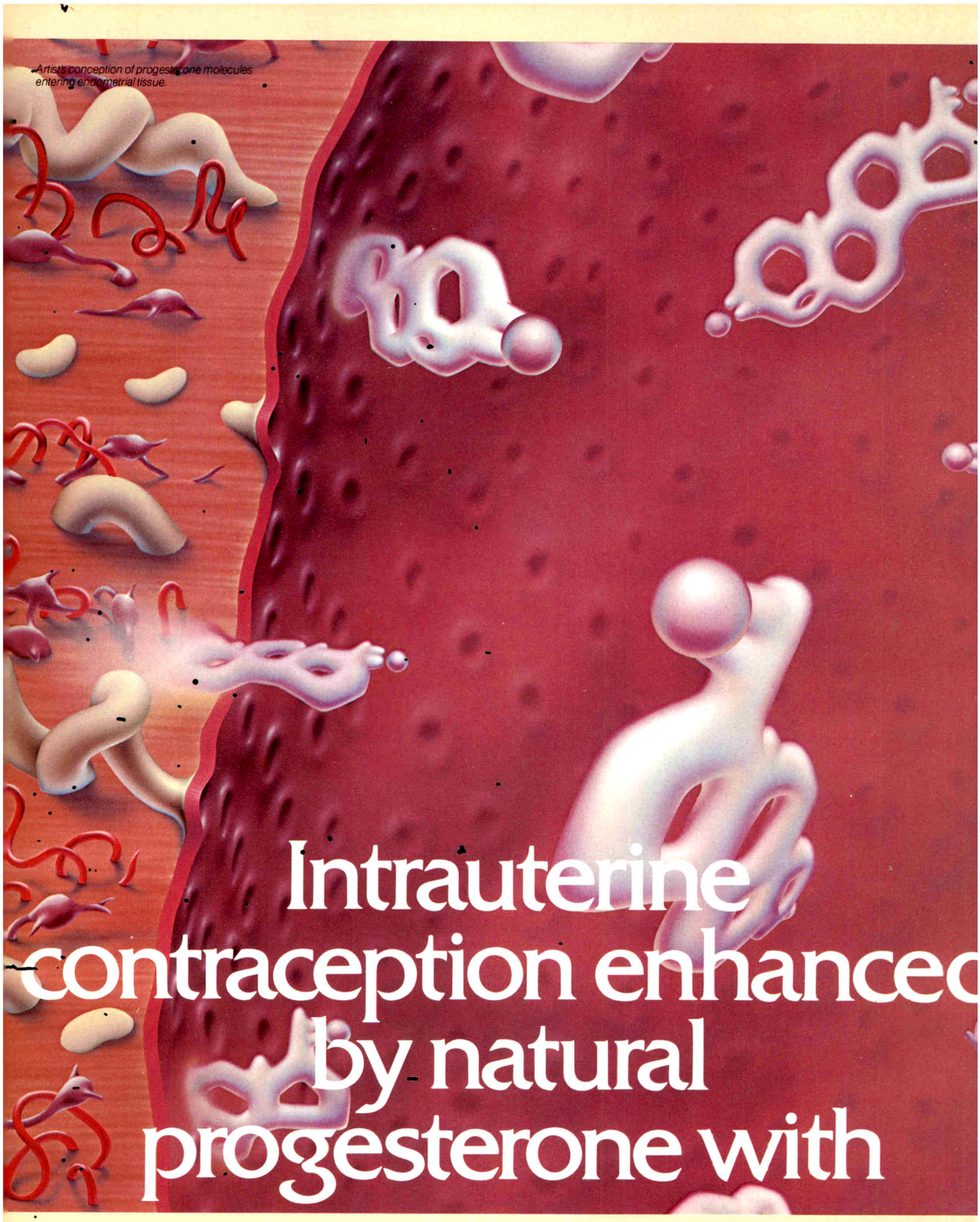


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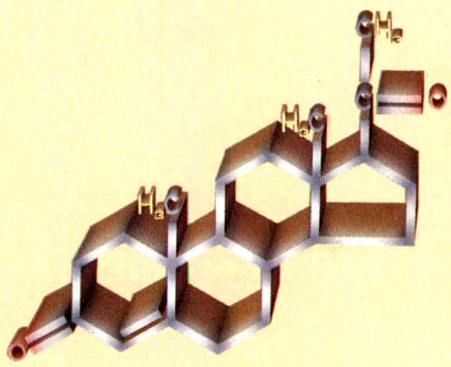
The natural ovarian hormone progesterone is placed to act continuously directly within the target organ — the uterus. Thus, only minute quantities of this rapidly catabolized hormone are needed.

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Contraceptive efficacy of the Progestasert® System enhanced by progesterone



WHY PROGESTERONE?

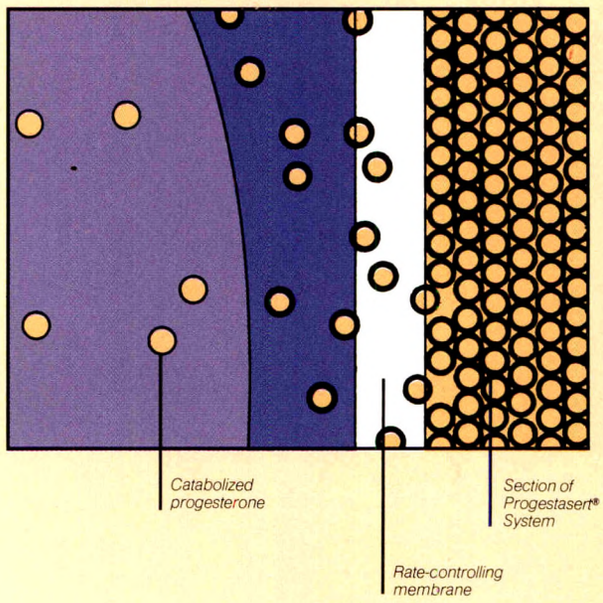
Progesterone—pregn-4-ene-3,20-dione ($C_{21}H_{30}O_2$)—is a naturally occurring female hormone, produced by the corpus luteum, adrenal cortex and placenta.

Progesterone is the contraceptive agent of choice for the Progestasert® System because:

- ☐ it is endogenous to the endometrium
- ☐ it has a short metabolic half-life
- ☐ it enhances the contraceptive effect at low concentrations when administered locally to the uterus
- ☐ it is rapidly catabolized by the endometrium
- ☐ it permits continuation of ovulation and, therefore, the length of the menstrual cycle is unaltered
- ☐ its effect on the endometrium reverses rapidly upon removal of the Progestasert® System.

CONTINUOUS MICRODOSAGE OF PROGESTERONE

The total quantity of progesterone contained in one Progestasert® System for one year's use is only 38 mg—less than the amount produced in one day by the corpus luteum during the latter part of the menstrual cycle. The amount of progesterone released daily from the System corresponds to a small percentage of the daily physiologic production.



A unique drug delivery system

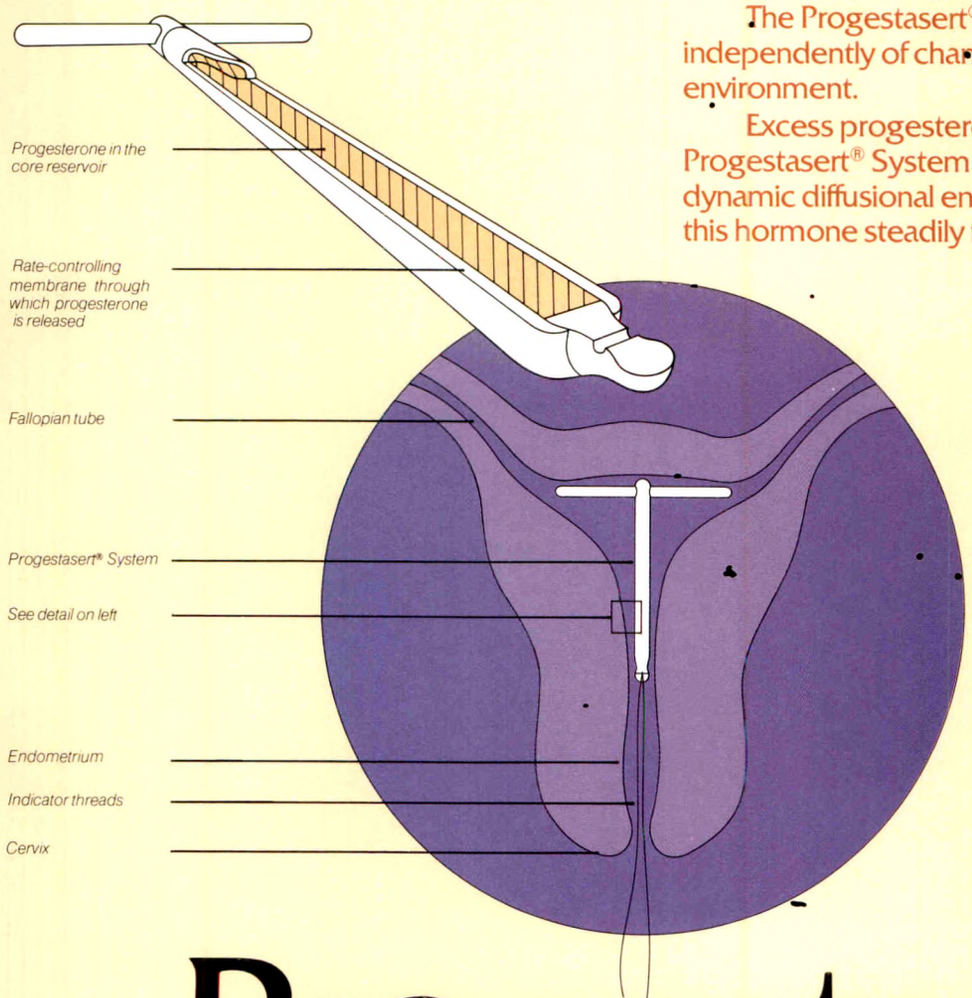
The Progestasert® System is a diffusional system designed to deliver microquantities of progesterone. The two key components are:

- ☐ a core reservoir of progesterone crystals with barium sulfate (for radiopacity) in medical-grade silicone oil
- ☐ an inert diffusional membrane of ethylene/vinyl acetate copolymer that surrounds the core reservoir and provides programmed control of progesterone release

When the Progestasert® System is placed in the uterine environment, progesterone molecules diffuse through the membrane into the uterine lumen at the average rate of 65 micrograms per day for one year.

The Progestasert® System functions independently of changes in the uterine environment.

Excess progesterone within the Progestasert® System provides the thermodynamic diffusional energy needed to release this hormone steadily for an entire year.



WHY THE T CONFIGURATION?

A T-shaped configuration was selected to contain the progesterone reservoir because this configuration has shown:*

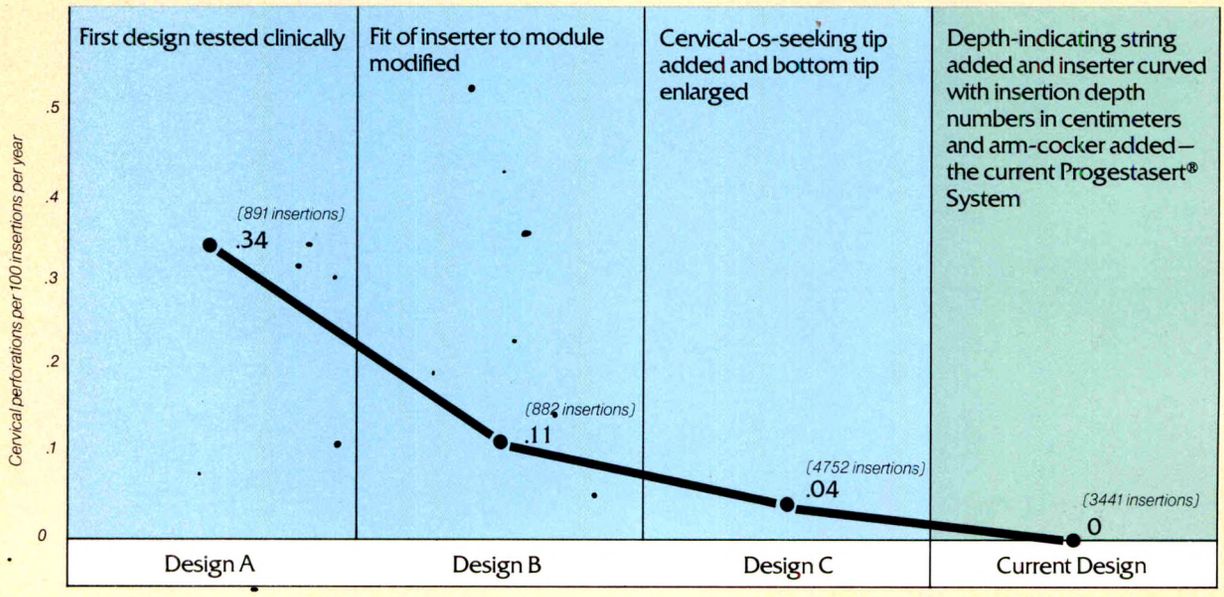
- ☐ easy insertion
- ☐ excellent retention by the uterus
- ☐ minimal symptomatic evidence of uterine irritation
- ☐ easy removal

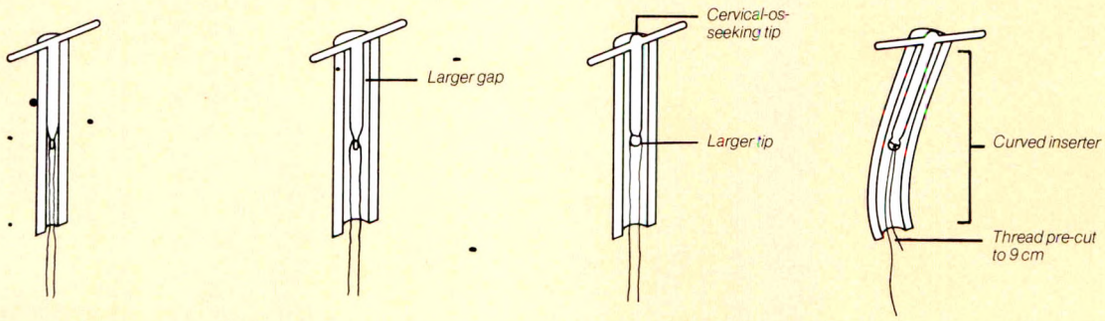
*Tatum HJ: Intrauterine Contraception. American Journal of Obstetrics and Gynecology, 112: 1000, 1972.

Progestasert®

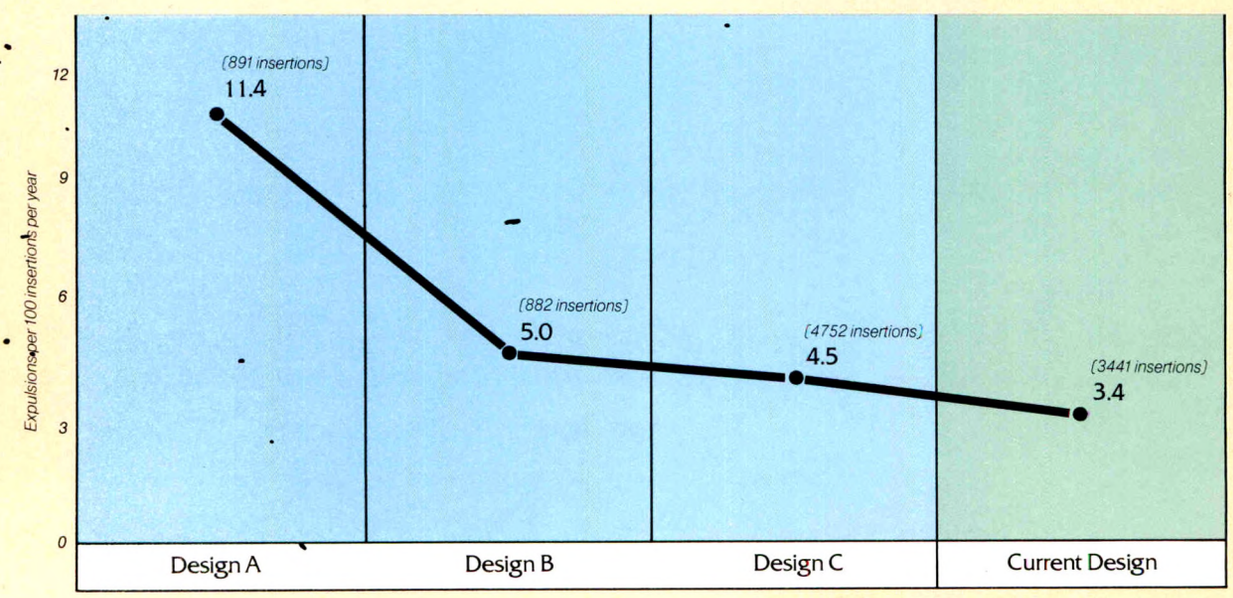
Intrauterine Progesterone Contraceptive System

CERVICAL PERFORATIONS REDUCED AS ALZA'S SYSTEM AND INSERTER DESIGN IMPROVED





EXPULSIONS REDUCED AS ALZA'S SYSTEM AND INSERTER DESIGN IMPROVED.



These graphs do not represent a comparison of inserters produced by different manufacturers. Rather, they depict stages in ALZA's development of the Progestasert® System inserter.

All numbers are expressed as events per 100 women per year.

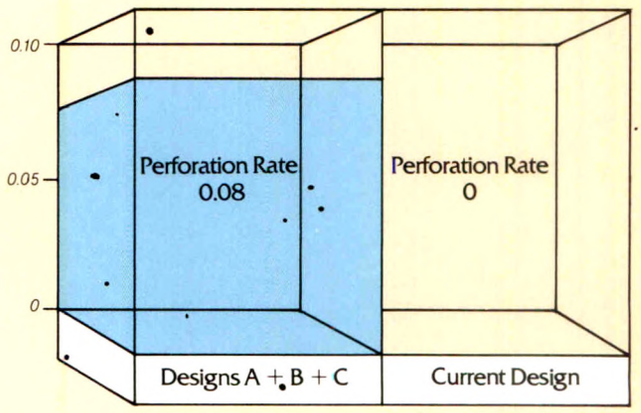
Inserter designed for safety and effective placement

As recently noted by Mishell, inserter design is increasingly recognized as important to the efficacy and safety of intrauterine contraceptive devices. "Proper placement...is of extreme importance for IUD effectiveness... (Thus), more attention should be directed toward the development of optimal IUD inserters than to changes in the IUD design."*

Some untoward effects with intrauterine devices—such as perforation and expulsion—in the past have been due to difficulties in placement. Since the technique of insertion and correct high fundal positioning play an important role in assuring contraceptive effectiveness and patient comfort and safety, much attention has been directed toward the development of the Progestasert® System inserter.

In controlled studies, evolutionary changes in the inserter and the Progestasert® System have demonstrated a decrease in the incidence of expulsions, cervical perforations, and uterine perforations which have paralleled these design changes. The incidence for both cervical and uterine perforations has decreased to the point that neither type of perforation has occurred with the 3,441 insertions with the final design after an average of more than nine months of exposure for these women.

UTERINE PERFORATIONS REDUCED AS ALZA'S SYSTEM AND INSERTER DESIGN IMPROVED



While these data reflect the importance of the design changes, the lack of perforations during these limited trials in no way suggests the Progestasert® System and its inserter will be trouble-free in general use. Perforations, both cervical and uterine, may occur with the Progestasert® System as its use becomes more widespread. The rate of perforation will depend upon anatomical and physiological variations of the women receiving the System and the care taken during the insertion process.

Progestasert®

Intrauterine Progesterone Contraceptive System

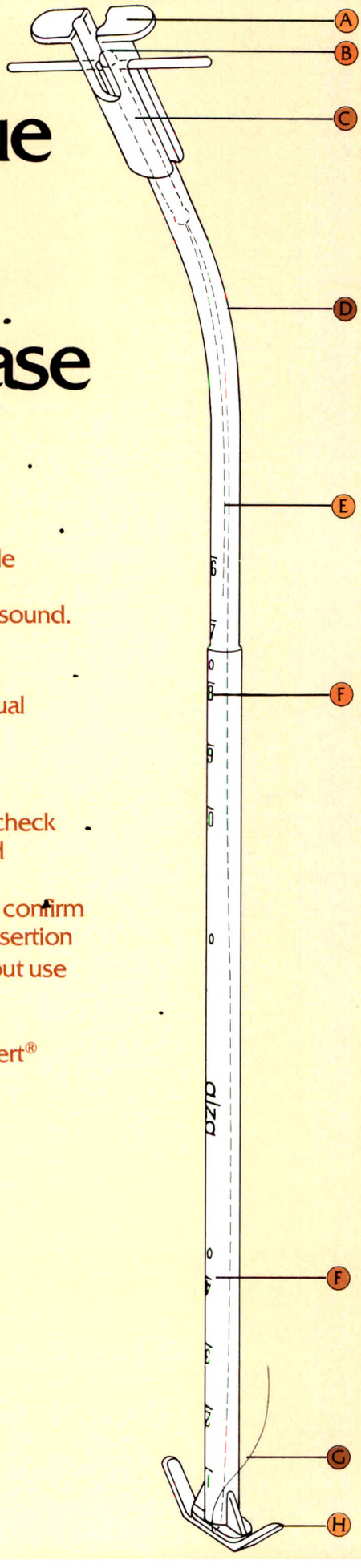
The System and its unique inserter-designed for safety and ease of insertion

The Progestasert® System inserter facilitates correct System placement while minimizing the risk of perforation, and is designed to function much like a uterine sound.

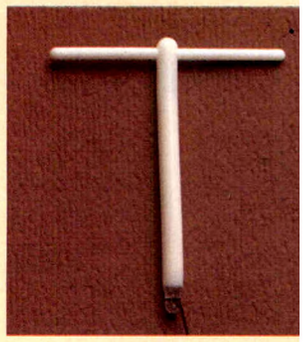
Features of the inserter:

- ☐ pre-curvature to facilitate insertion
- ☐ malleability for adapting to the individual uterus
- ☐ dual checking mechanism for correct placement
  - — centimeter markings on inserter to check conformity with previously sounded uterine depth
  - — indicator thread length designed to confirm proper System position following insertion
- ☐ high fundal placement achieved without use of plunger or push-rod

Comprehensive illustrated insertion instructions are a part of every Progestasert® System package.



Actual Size



A. Arm-cocker allows the arms of the T to be folded aseptically, using one-handed technique just prior to insertion.

B. Cervical-os-seeking tip helps guide the System into the cervical canal, making insertion easier and more comfortable.

C. Vertical stem of the System designed with flexibility and rounded tip to minimize cervical perforation risk.

D. Curved portion of inserter designed to conform to anatomic configuration of most cervical-uterine cavities. Malleability allows accomodation to variations in uterine anatomy.

E. Indicator monofilament thread, extended 9 cm from the top of the System, verifies correct placement a insertion.

F. Graduations (in centimeters to determine uterine position and depth, and to confirm correct System placement.

G. Retainer monofilament thread extends the length of the inserter and is anchored by the thread-retaining plug to retain the System in the inserter.

H. Thread-retaining plug is removed to release the System in the uterus.

Safety studies

Clinical safety studies demonstrated:

- No extrauterine metabolic or endocrine effects in humans*
- Endometrial changes induced by the Progestasert® System disappear within weeks after System removal
- Rapid return to fertility confirmed in women discontinuing use of the Progestasert® System in the hope of becoming pregnant. The cumulative conception rate among 154 women was 49 per 100 women pregnant at 3 months after removal, and 76 per 100 women at 12 months after removal. These rates of return to fertility are similar to those reported following removal of an IUD.

Effective contraception for one year from a single placement

The Progestasert® System functions unattended for one year at a continuous release rate of 65 micrograms of progesterone per day, eliminating reliance on daily hormone administration.

Yearly removal and placement can coincide with annual visits for physical examination and Pap smear, as encouraged by the American College of Obstetrics and Gynecology. (Currently accepted practice used by recognized authorities, reported in ACOG Technical Bulletin #29, February, 1975, p 7.)

Progestasert®

Intrauterine Progesterone Contraceptive System

Some clinical aspects

Data were collected in 40 clinics involving 5,495 women over a period of 45,848 woman-months. These trials were designed to obtain data that would predict the true use-effectiveness of the System.

HIGHLY EFFECTIVE INTRAUTERINE CONTRACEPTION

Clinical results have demonstrated pregnancy rates of 1.9† in parous patients and 2.5† in nulliparous patients. A similar T-shaped unit, but without progesterone in the stem, produced a pregnancy rate of 18.3†.

HIGH DEGREE OF ACCEPTABILITY

Clinical studies have demonstrated a high continuation rate. Approximately 8 of every 10 parous and 7 of every 10 nulliparous women continued use of the Progestasert® System for a full year.

SHAPE PROVIDES EXCELLENT RETENTION

Expulsion rates of 3.1† and 7.5† were observed for parous and nulliparous women respectively.

INSERTER PROVIDES SAFE PLACEMENT

No uterine perforations have occurred with 3,441 insertions using the current pre-curved, malleable inserter during controlled clinical trials. This should not be interpreted as a guarantee that perforations will not occur with the Progestasert® System.

	Parous	Nulliparous
PREGNANCY RATE	1.9	2.5
EXPULSIONS	3.1	7.5
CONTINUATION RATE	79.1	70.9

Features and benefits

- ☐ Contraception provided by progesterone, identical to the natural ovarian hormone
- ☐ No known systemic hormonal effects*
- ☐ Continuous delivery of progesterone in microgram amounts/day
- ☐ Effective contraception for one year from one System placement
- ☐ High degree of patient acceptability
- ☐ Excellent uterine retention
- ☐ Minimal reliance on patient compliance during its year of use when compared to extrauterine contraceptives
- ☐ Prompt return to pre-insertion fertility following System removal
- ☐ Inserter designed for safety and ease of insertion

Candidates for the Progestasert® System

THE NEW CONTRACEPTIVE USER

The natural progesterone contraception provided by the Progestasert® System without systemic hormonal effects, along with minimal dependence on patient compliance, small unit size, and easy insertion, make the Progestasert® System an attractive first contraceptive for many women.

THE NONCOMPLIER

The daily regimen of pill-taking proves beyond the capability of some patients for whom hormonal contraception is preferable. The Progestasert® System reduces requirements for patient compliance to once-a-year replacement.

THE PATIENT DISCONTINUING ORAL CONTRACEPTIVES

Patient-initiated—the Progestasert® System offers the opportunity to continue hormonally enhanced contraception without the adverse systemic hormonal effects* that cause many women to dicontinue oral contraceptives.

Physician-initiated—some physicians consider it important for the patient to discontinue oral contraceptive use until the body regains its normal hormonal balance. Some physicians discontinue the Pill permanently. Recent studies strongly suggest that oral contraceptive users are at greater risk of developing coronary thrombosis than nonusers. The Progestasert® System, with its lack of adverse systemic hormonal effects,* can be the desired alternative contraceptive for former users of the Pill.

THE PATIENT DISCONTINUING OTHER CONTRACEPTIVE METHODS

The inconvenience and lower efficacy rates of diaphragms and condoms make the Progestasert® System an attractive alternative for the patient desiring intrauterine hormonal contraception without systemic hormonal effects.*

THE IUD PATIENT DUE FOR REINSERTION

For those patients already using some form of intrauterine contraception, the Progestasert® System, designed for safety, offers an effective alternative to their present IUD and may be considered at reinsertion time.

Progestasert®

Intrauterine Progesterone Contraceptive System

Ordering Information

IF YOU WOULD LIKE FURTHER INFORMATION ABOUT ALZA, THERAPEUTIC SYSTEMS, THE PROGESTASERT® SYSTEM, DETAILED INSERTION INSTRUCTIONS OR WOULD LIKE TO ORDER PROGESTASERT® SYSTEMS FOR YOUR PRACTICE, CALL THE FOLLOWING TOLL-FREE NUMBER: (800) 227-9953 OR WRITE TO ALZA PHARMACEUTICALS, 3170 PORTER DRIVE, PALO ALTO, CALIFORNIA 94304.

Progestasert®

Intrauterine Progesterone Contraceptive System

Release rated 65 μg/day progesterone for one year.

DESCRIPTION

The Progestasert® Intrauterine Progesterone Contraceptive System is a white, T-shaped unit constructed of ethylene/vinyl acetate copolymer (EVA) containing titanium dioxide. The 36 mm tubular vertical stem of the T contains a reservoir of 38 mg of progesterone, USP, together with barium sulfate, USP, for radiopacity; both are dispersed in medical grade silicone oil. The 32 mm horizontal crossarms are solid EVA. Two monofilament nylon indicator/retrieval threads are fastened to the base of the T stem. The Progestasert® system is packaged sterile within its curved, malleable inserter. Progesterone is released from the system at an average rate of 65 μg/day for one year by membrane-controlled diffusion from the reservoir. None of the inert ingredients of the reservoir or membrane—barium sulfate, silicone oil, titanium dioxide, or EVA—is released from the system.

ACTION

Available data indicate that the contraceptive effectiveness of the Progestasert® system is enhanced by its continuous release of progesterone at an average rate of 65 μg/day into the uterine cavity. The mechanism by which progesterone enhances the contraceptive effectiveness of the T is local, not systemic. The concentration of luteinizing hormone, estradiol, and progesterone in systemic venous plasma follow regular cyclic patterns indicative of ovulation during use of the Progestasert® system. Blood chemistry studies related to liver, kidney, and thyroid function also reveal no changes.

During use of the system, the endometrium shows progestational influence. Following removal of the system, the endometrium rapidly returns to its normal cyclic pattern and can support pregnancy. The local mechanism by which continuously released progesterone enhances the contraceptive effectiveness of the T has not been conclusively demonstrated. The hypotheses that have been offered are: progesterone-induced inhibition of sperm capacitation or survival, and alteration of the uterine milieu so as to prevent nidation.

INDICATION

The Progestasert® system is indicated for contraception.

CONTRAINDICATIONS

Pregnancy or suspicion of pregnancy; abnormalities of the uterine cavity; acute pelvic inflammatory disease or history of repeated pelvic inflammatory disease; postpartum endometritis or infected abortion in the past 3 months; uterine or cervical malignancy; unexplained genital bleeding until suspicion of cancer is ruled out; acute cervicitis.

WARNINGS

A. Pregnancy

1. Septic abortion. Recent reports have indicated an increased incidence of septic abortion, associated in some instances with septicemia, septic shock and death in patients becoming pregnant with an intrauterine device (IUD) in place. In some cases, the initial symptoms have been insidious and not easily recognized. If pregnancy should occur with a Progestasert® system in situ, it should be removed if the thread is visible or, if removal proves to be or would be difficult, interruption of the pregnancy should be considered and offered as an option. If the patient elects to maintain the pregnancy and the system remains in situ, she shall be warned of the increased risk of sepsis and be followed with close vigilance.
2. Congenital anomalies. Systemically administered sex steroids, including synthetic progestational agents, have been associated with an increased risk of congenital anomalies including cardiovascular anomalies. Such effects have not been reported with progesterone itself, which is produced in large concentrations during pregnancy.
3. Ectopic pregnancy. A pregnancy that occurs while a patient is wearing an IUD is much more likely to be ectopic in nature. Therefore, such patients in whom pregnancy occurs should be carefully evaluated.

B. Severe Pelvic Infection

Occasionally serious pelvic infection may occur with an IUD in situ. Under such circumstances the IUD should be removed, appropriate bacteriological studies done, and appropriate antibiotic treatment instituted.

C. Perforation

Perforation of the uterus may occur. If this occurs, the device should be removed. Local inflammatory reaction with abscess formation is a possibility if an IUD is left in the abdomen. Cervical perforation, often asymptomatic, has also been reported. If such occurs, the device should be removed.

PRECAUTIONS AND ROUTINE EXAMINATIONS

A. Patient Examination

The physician should make a determination that the patient is not pregnant. The possibility of insertion in the presence of an existing undetermined pregnancy is reduced if insertion is performed during or shortly following a menstrual period. The system should not be inserted postpartum or postabortion until involution of the uterus has been completed. Prior to insertion, a pelvic examination, pap smear, and gonorrhea culture should be done. Sound the uterus prior to insertion and exercise care to avoid perforation. Do not use excessive force. The patient should be told that some bleeding or cramps may occur during the first few days after insertion. If these symptoms continue or are severe, she should report to her physician. She should be cautioned not to pull on the threads, which would displace the system.

B. Requirements for Continuation and Removal

The Progestasert® system must be replaced each year. There is no evidence of decreasing contraceptive efficacy with time before one year, but the contraceptive effectiveness of the Progestasert® system after one year has yet to be established. Therefore, the patient should be informed of the known duration of the contraceptive efficacy and be advised to return in one year for re-examination, removal and possible reinsertion.

The Progestasert® system should be removed for the following medical reasons: Menorrhagia and/or metrorrhagia producing anemia; uncontrolled pelvic infection; intractable pain, often aggravated by intercourse; pregnancy, if the thread is visible; uterine or cervical malignancy; uterine or cervical perforation; an increase in length of thread extending from the cervix, or any other indication of partial system expulsion.

C. Continuing Care of Patients Using the Progestasert® System

Patients should be re-examined and evaluated within three months after Progestasert® system insertion. Routine annual examination, removal of the Progestasert® system, and reinsertion of a new Progestasert® system along with appropriate medical and laboratory evaluation should be carried out.

ADVERSE REACTIONS

Reported adverse reactions include: Endometritis, spontaneous abortion, septic abortion, septicemia, perforation of uterus and cervix, pelvic infection, cystic masses in pelvis, abdominal adhesions, intestinal obstruction, cervical erosion, vaginitis, leukorrhea, cystitis, pregnancy, ectopic pregnancy, uterine embedment, difficult removal, complete or partial expulsion, intermenstrual spotting, prolongation of menstrual flow, anemia, amenorrhea or delayed menses, pain and cramping, backaches, dyspareunia, leg pain or soreness, weight loss or gain, nervousness, bradycardia and syncope secondary to insertional pain.

DOSAGE AND ADMINISTRATION

A single Progestasert® system is to be inserted into the uterine cavity (see Precautions section). Present information indicates that the contraceptive effectiveness of the system is retained for one year and the system must be replaced one year after insertion.

HOW SUPPLIED

Available in cartons containing six sterile systems (Progestasert® system with an inserter).

CLINICAL STUDIES

Different event rates have been recorded with the use of different contraceptive methods. Inasmuch as these results are usually derived from separate studies conducted by different investigators in several population groups, they cannot be compared with precision. Furthermore, event rates tend to be lower as clinical experience is expanded, possibly due to retention in the clinical study of those patients who accept the treatment regimen and do not discontinue due to adverse reactions or pregnancy. In clinical trials sponsored by Alza with the Progestasert® system, use-effectiveness was determined for parous and nulliparous women by the life table method. (Rates are expressed as events per 100 women through 12 months of use.) This experience is based on 45,848 woman-months of use with 5,495 women, of whom about 25% were nulliparous; 2,157 women completed 12 months of use.

	Parous	*Nulliparous*
Pregnancy	1.9	2.5
Expulsion	3.1	7.5
Medical Removals	12.3	16.4
Continuation Rate	79.1	70.9

CAUTION

Federal law prohibits dispensing without prescription.

alza

Fetal membrane dynamics

W. A. SCOGGIN, M.D.

Augusta, Georgia

The validity of the concepts of exchange and net flow and of the method used to measure them was proved by utilizing a standard artificial membrane. The exchange rates across the amniochorion of D- *and* L-*isomers of various amino acids have been measured. They do not differ. Rates of exchange of water, glucose, and mannitol across the umbilical cord were determined and were noted to be considerably slower than the rates for these substances across the amniochorion.*

MYERS AND Prestwood[1] have stated, "when the progress of a simple isotopic reaction is followed in a homogeneous stable system, the rate of appearance of radioactive atoms in the initially untagged reactant follows a simple exponential law. The exponential law is followed regardless of the mechanism by which the exchange occurs, the number of exchangeable atoms in each species, or the concentration of the radioactive atoms." McKay[2] first derived this law, and my colleagues and I[3] have previously utilized a derivation[1] of McKay's formula for calculation of rate of exchange in a homogeneous, stable system:

$$Rt = \frac{-AB}{A + B} \; {}^{e}\log \; (1 - F)$$

$$F = \frac{S_b - S_{b0}}{S_{b\infty} - S_{b0}}$$

to measure exchange and net flow of water across the human, term, amniochorionic membrane in vitro. There exists controversy regarding the concept of exchange and net flow versus diffusion. The first portion of this investigation was undertaken to test the validity of the use of the derivation of McKay's formula.

There is abundant evidence that the placental membrane possesses the capability of selective or facilitative transport of some substances, whereas simple diffusion can account for the transfer of others. Page, Glendening, Margolis, and Harper,[4] and Kelly, Hutchinson, Friedman, and Plentl[5] have shown that the naturally occurring amino acid, L-histidine, traverses the primate placenta more rapidly than does the D-isomer. While I have no evidence of an active process involved in passage of either solute or solvent across the term amniochorion, the second phase of the study was designed to ascertain whether or not this membrane could discriminate between D- and L-amino acids as determined by rates of passage of the isomers. Further, a comparison was made of the relative rates of transfer of amino acids of increasing molecular weights.

In addition to the placenta and the amniochorion, the umbilical cord represents a potential and possibly significant site of exchange—not between mother and fetus but between fetal blood and amniotic fluid. The third portion of this study involved an appraisal of transfer through the umbilical cord.

Methods

If our calculation of unidirectional rate of exchange is erroneous and represents rate of diffusion of radioactive isotope in response to the gradient of the tracer across the membrane, then changing this gradient should result in an altered transfer rate. On the other hand, changing the total concentration of test substance, which is present in equal concentrations on the two sides of the membrane, without altering the gradient of the isotope, should not affect the rate found. If the calculation is valid, the reverse should be true.

The previously described two-chamber system[3] was employed. In order to obviate errors which might be produced by swelling or shrinkage of cells in the amniochorion resulting from varying the molarity of

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Table I. Rates of exchange of L-methionine across a cellulose membrane

Total L-methionine (mM./L.)	*^{14}C–L-methionine (μCi./L.)*	*Rate of exchange (uM/hr./cm.^{2})*
10	25	17.5
10	50	18.5
10	100	17.8
20	25	36.1
20	50	36.5
20	100	38.6
30	25	56.4
30	50	60.4
30	100	58.4

Table II. Rates of exchange of L-methionine (10 mM. per liter) across a cellulose membrane, with ^{14}C tracer on one side of the membrane disk and ^{3}H tracer on the other

	Rate of exchange (^{14}C data) (uM/hr./cm.^{2})	*Rate of exchange (^{3}H data) (uM/hr./cm.^{2})*
	19.3	19.7
	17.8	21.0
	19.4	15.4
	16.1	22.5
	20.1	18.8
	16.2	16.9
Average	18.2	19.1

the bathing solution, a cellulose membrane (Arthur H. Thomas Company No. 4465-A2 Dializer Tubing) was utilized. This membrane possesses a uniform thickness of 0.04 mm. and an average pore size of 48 Å. The membrane was placed over the open end of the small chamber (70 ml. volume) and fastened with rubber bands. The small chamber was suspended in the large compartment (1,000 ml.) and both chambers were filled simultaneously with Locke's solution to which had been added an appropriate amount of test substance (L-methionine).

Mixing of solutions was assured by bubbling a gas mixture, 95 per cent oxygen and 5 per cent carbon dioxide, through both chambers. Samples were withdrawn from both chambers at the beginning of each experiment and at 30 minute intervals for a period of 2 hours. Radioactivity in each sample was determined with a Unilux II liquid scintillation spectrometer.

In 45 experiments, the solution added to the small chamber contained a measured amount of ^{14}C–L-methionine as tracer. In 15 of these experiments the total concentration of L-methionine was 10 mM. per liter. In 15, 20 mM. per liter of L-methionine was used; in the other 15, 30 mM. per liter. Within each group of 15 tests, the amount of tracer was varied (Table I). Thus, total concentration of the amino acid and amount of radioactive tracer were varied together and independently. In an additional six experiments ^{14}C–L-methionine (25 μCi per liter) was placed in the solution in one chamber and ^{3}H-L-methionine (125 μCi per liter) in the other, with a total concentration of 10 mM. per liter of L-methionine (Table II). The purpose of this group of experiments was to determine whether or not exchange would occur in both directions across the membrane.

Table III. Rates of exchange of various amino acids, in a concentration of 10 mM. per liter, across the human term amniochorionic membrane

Amino acid	*No. of experiments*	*Rate of exchange (uM/hr./cm^2)*
Glycine	18	10.3
D-Alanine	13	6.5
L-Alanine	13	7.0
D-Valine	10	6.1
L-Valine	10	7.7
D-Leucine	10	7.7
L-Leucine	10	7.1
D-Methionine	9	6.5
L-Methionine	9	6.3
L-Histidine	10	7.8
D-Phenylalanine	11	6.4
L-Phenylalanine	11	5.9

In the second phase of the study fresh amniochorionic membranes were used in the two-chamber arrangement as described above. Various amino acids (Table III), in a concentration of 10 mM. per liter, were added to Locke's solution in the two compartments and 25 μCi per liter of the appropriate ^{14}C isotope were placed in the solution in the small chamber. In each instance, the Locke's solution was buffered to the isoelectric point of the amino acid being used. Sampling and determination of radioactivity in each sample were performed as before.

The third part of the investigation consisted of measurement of exchange through umbilical cords. Immediately after delivery, sections of term umbilical cord, varying from 12 to 42 cm. in length, were excised and examined for the presence of three vessels and the absence of apparent defects, and the vessels were cannulated with modified thin-walled 14 gauge needles. The modification consisted of cutting off the pointed ends of the needles, rounding off the edges, and securing two epoxy beads on the shafts. In the first 94 experiments, only the veins were cannulated. The

Table IV. Rates of exchange of glucose (5.5 mM. per liter) and of mannitol (5.5 mM. per liter) across the cord and across the amniochorion

Substance	*Direction of measured exchange*	*Rate of exchange (uM/hr./cm.²)*
Umbilical cord:		
Glucose	Vein → bath	0.326
	Bath → vein	0.187
Mannitol	Vein → bath	0.348
	Bath → vein	0.130
Amniochorion:		
Glucose	Amnion → chorion	4.33
	Chorion → amnion	4.29
Mannitol	Amnion → chorion	3.24
	Chorion → amnion	3.50

cannulas were connected by means of tubing to a reservoir containing 1,000 ml. of Locke's solution maintained at 37° C. The Locke's solution was circulated through the umbilical vein at a flow rate of 150 ml. per minute by means of a Harvard Apparatus Peristaltic Pump while the cord was immersed in a bath containing 500 ml. of Locke's solution at 37° C. Transfer of glucose, 5.5 mM. per liter of solution, with ^{14}C-glucose as tracer being placed alternately in the perfusate reservoir and in the immersion bath, was measured in 22 experiments. Samples were obtained from both reservoir and immersion bath at the start of each experiment and at 30 minute intervals for 2½ hours. Radioactivity in each sample was determined as described above, and exchange calculated by use of the derivation of McKay's law. Because of the possibility that glucose might be metabolized by cells in the cord, its rate of exchange was compared to that of mannitol as determined in 24 experiments with the same protocol. These exchange rates were then compared with rates of transfer of glucose and of mannitol across human term aminochorionic membranes. The latter measurements were performed with the two-chamber system in 48 experiments.

Rates of exchange of water, in two different concentrations, across the umbilical cord were determined. In 24 experiments, ordinary Locke's solution, containing 55 moles of water per liter (equivalent to water concentration in amniotic fluid), was utilized. In an additional 24 experiments, the Locke's solution was modified by the addition of sucrose so that the concentration of water was 46 moles per liter, approximating water concentration in fetal blood. In all of these experiments tritiated water was used as the isotopic tracer for movement of water.

After considerable trial and error, we were able finally to cannulate the umbilical arteries successfully. The perfusate was then pumped in a pulsatile flow at 220 ml. per minute through the two arteries simultaneously and returned via the single umbilical vein, in which flow was nonpulsatile, to the reservoir for recirculation. With the use of this model in 10 experiments, transfer of water across the umbilical cord was again determined.

Results

The formula (derived by Myers and Prestwood[1] from McKay's[2] law) used to calculate rate of exchange is given in the beginning of this article. According to the authors, the formula is valid for isotopic exchange in a stable homogeneous system. In the formula, R is the constant rate of exchange of molecules of measured substance; t is time; A is concentration of the substance in the compartment initially containing the isotopic tracer; B is concentration of the substance in the other compartment; and F is the fraction exchange. S_b represents specific activity at time t in the compartment with the initially untagged substance and the subscripts 0 and ∞ refer to zero time and at isotopic equilibrium, respectively.

In Table I the calculated rates of exchange of L-methionine across a cellulose membrane in three different concentrations are shown. For each concentration of L-methionine, the amount of isotopic tracer was also varied. The rate of exchange of L-methionine was dependent upon total concentration of the amino acid and independent of the amount of isotopic tracer.

Table II depicts the rates of exchange of L-methionine, in a concentration of 10 mM. per liter, across the cellulose membrane in six experiments in which unidirectional exchange was determined in both directions by using two different isotopic tracers. As shown, exchange occurred in both directions and rate of exchange in one direction across the membrane is equal to that in the other direction in a stable homogeneous system.

Table V. Rates of exchange of water across umbilical cords, vein alone perfused, with tritiated water as tracer

Concentration of water in Locke's solution (M/L.)	*Direction of measured exchange*	*Rate of exchange (M/hr./cm.²)*
55	Umbilical vein → bath	0.019
55	Bath → umbilical vein	0.018
46	Umbilical vein → bath	0.016
46	Bath → umbilical vein	0.014

Table VI. Rate of exchange of water across umbilical cords, arteries and vein cannulated

Concentration of water (M/L.)	*Direction of measured exchange*	*Rate of exchange (M/hr./cm.²)*
55	Vessels → bath	0.031
55	Bath → vessels	0.033

Having thus demonstrated that the formula for calculation of constant rate of exchange is valid, and that exchange does occur in both directions across a membrane, I proceeded with further measurement of exchange across human fetal membranes. In Table III are given rates of exchange of various amino acids, including D- and L-isomers where practicable. The amino acids studied are listed in progressive order of molecular weight. There is no difference between rates of exchange of D- and L-isomers. Except for glycine, the smallest amino acid, there is no constant variation in exchange rate relative to molecular size.

Initiating a study of transfer through the umbilical cord, rates of exchange of the solutes glucose and mannitol were determined. Table IV shows the rates for these two substances in each direction across the cord. For comparison, rates for glucose and mannitol transfer across the amniochorionic membrane were measured and are listed in the table. Measured transfer rates of glucose and mannitol from umbilical vein to bath are considerably greater than those in the opposite direction. Rates of passage of these substances across the amniochorion are approximately 10 times faster than through the cord.

In Table V are shown calculated rates of exchange of water between umbilical vein and immersion bath with regular and modified Locke's solution. The differences between rates of water transfer from vein to bath and from bath to vein are consistent with—but of much less magnitude than—was found with glucose and mannitol. The 10 experiments in which both arteries and vein were cannulated and perfused provided a calculated rate of water exchange given in Table VI, approximately 1.7 times faster than with the vein alone cannulated.

Comment

The fact that two- and threefold increases in total concentration of an amino acid resulted in two- and threefold increases in calculated rate of exchange across a membrane represents experimental confirmation of the validity of McKay's law and of the formula derived therefrom. Since exchange rate varies with total concentration of a substance and is not affected by a threefold increase in amount of isotopic tracer, clearly exchange is not the same as diffusion. If the radioactive isotope diffused through the membrane in response to a gradient of the isotope between the two compartments it might be expected to diffuse more rapidly with a higher gradient rather than with a greater concentration of its nonradioactive isotope which had no gradient across the membrane. Those experiments in which different radioactive isotopes were placed on opposite sides of the membrane demonstrate that exchange does occur in both directions and at equal rates in a homogeneous system.

Battaglia and Hellegers[6] reported that permeability constants for several oligosaccharides and pentoses, as determined by their transfer through the human chorion laeve in vitro, were not significantly different from one another. The finding reported here that the human amniochorion does not demonstrate stereoisomeric specificity relative to transfer of D- and L-amino acids is further evidence for passive transfer mechanisms through this membrane. There is to date no evidence of active transport across this membrane of any substance studied.

Glucose, mannitol, and water were all found to traverse the umbilical cord in vitro. The fact that transfer rates of these substances through the cord are slower than those across the amniochorion may be due to difference in thickness of the two membranes. The observation that rate of exchange of water was 1.7 times greater in those experiments in which all three cord vessels were perfused than in those in which only

the vein was cannulated demonstrates that the design of the experimental model is important. Because of this variability, the data presented do not permit mathematical extrapolation to the in vivo situation. They do strongly suggest, however, that water may move from amniotic fluid through the cord to fetal blood and that small amounts of glucose may cross in the opposite direction.

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Discussion

DR. W. L. JOHNSON, Buffalo, New York. Dr. Scoggin has presented a series of experiments testing the hypothesis of active transport across the human chorioamnion. His results fail to show evidence of active transport in the system used. He has given us firm evidence that the isotopic technique of measuring transfer rates of substances across a standard membrane is valid by demonstrating in a series of experiments that the rate of exchange of L-methionine is dependent upon total concentration of amino acid and independent of the isotopic tracer used.

The biologic membrane (human chorioamnion) was stretched between two plastic chambers and rates of exchange measured of amino acids comparing the dextro- and levo- isomers. Other investigators have reported more rapid transfer of the naturally occurring L-histidine than the D- isomer. The concept of active transport would explain the increased concentration of amino acids in the fetal circulation compared to that in the maternal circulation. Dr. Scoggin's results fail to show any difference between transfer rates of the D- and L- isomers used, however, specific comparison was not made between D- and L-histidine. Unless histidine reacts or metabolizes differently from the other amino acids, one would anticipate that it would behave similarly to the others compared in this system. Is there a specific reason why histidine was not tested?

A standard nonviable membrane would be expected to function as a simple diffusion membrane without active transport. A living biologic membrane may facilitate active transport within the same in vitro system. Is there evidence available that the membrane at the completion of these experiments is alive and metabolically active? Tissue experiments using a Warberg apparatus would give us assurance that these rates are being measured in a living system and not a dead membrane simply acting as an agent for passive diffusion.

Dr. Scoggin's third set of experiments, that of transfer rates across the umbilical cord, showed all substances tested to cross slower than across the chorioamnion. Since the rate-controlling factors across a membrane are those of pore size, surface area, membrane thickness, molecular size and charge, concentration gradient, and hydrostatic pressure, I would agree that the lower transfer rates in this system probably are due to increased membrane thickness. May not the increased rate of transfer from umbilical vessels to bath be explained by the increased hydrostatic pressure used to pump solution through the vessels? Was this pressure differential measured and could it account for the differences in exchange rates? Similarly, may not the increased exchange noted when all three vessels were used compared with the experiments where one vessel was used be explained by the increase in surface area of the vessel walls, thereby increasing the rate of transfer in this system?

Dr. Scoggin is certainly to be congratulated on this work and on increasing our knowledge about this important topic of fetal membrane dynamics.

DR. RALPH M. WYNN, Chicago, Illinois. Studies of maternofetal transfer have largely neglected the paraplacental membranes or, at best, included the data with those relevant to the main placental structures. Nevertheless, advances in perinatal medicine have heightened our awareness of the importance of these membranes and their associated fluids. In this respect, Dr. Scoggin's paper is both timely and significant.

The present study attempts to distinguish active from passive diffusion and diffusion in general from total exchange. I agree with Dr. Scoggin's major conclusions but merely raise a few questions about the experimental design and the interpretation of the data.

In each set of three experiments, the values of S_{b_∞} must be in the ratios of 1:2:4 because the quantities of tracer are, respectively, 25, 50, and 100 μc per liter.

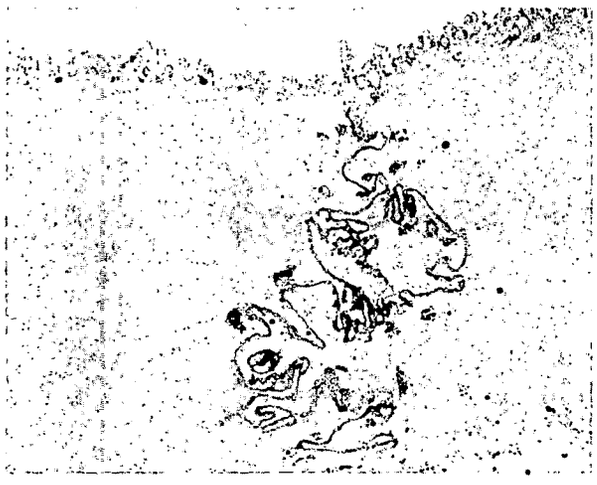


Fig. 1. Amnion incubated with adenosine monophosphate. Reaction products are deposited lightly on microvillous surface and heavily on lateral intercellular membranes.

Since the term

$$\left(1 - \frac{S_b}{S_{b\infty}}\right)$$

is nearly constant in all experiments, the ratios of S_b in each set of experiments must also be 1:2:4. Since doubling the concentration of the tracer doubles its rate of transfer, the tracer thus appears to move on its own concentration gradient. The formula is incomplete, however, in that it lacks a constant factor for conversion of the fractional transfer of tracer to total rate of exchange of all molecules in micromoles per hour per square centimeter. Thus

$$\log_e \left(1 - \frac{S_b}{S_{b\infty}}\right)$$

should be approximately −3.7K.

The data purport to show that the rate of transfer is independent of the concentration of tracer but proportional to the concentration of untagged molecules. The calculations that I have just made, however, show that the transfer of tracer does depend on its concentration. Why then are the calculated rates of transfer constant in each of the three experiments in each set? The answer is probably because $S_b/S_{b\infty}$ is a ratio that remains constant, with S_b doubling as $S_{b\infty}$ doubles. Thus the design of the experiment does not provide an unequivocal distinction of diffusion from total exchange. This answer could be provided by a calculation of the rate of appearance of the tracer in the initially unlabeled compartment in relation to the quantity of tracer used.

Dr. Scoggin's data showing equal rates of transfer in each direction of radioisotopes and of optical isomers are consistent with passive diffusion. What then is the mechanism for maintaining the hypotonicity and low concentration of sodium in the amnioic fluid at the same time as the high concentrations of creatinine, urea, and urate in late pregnancy? Simple diffusion should lead to equilization of concentrations between amnioic fluid and maternal blood unless the rate of input into the amnioic fluid exceeds the rate of diffusion.

Some years ago, we localized, by electron microscopy, nucleoside triphosphatases to the amnionic epithelium (Fig. 1). These enzymes, however, are normally associated with active transport. If the amnion is devoid of active transfer, its sodium pumps, which are ATPase-related, may function in the metabolism of the individual cell rather than in transfer across the full thickness of the epithelial membrane.

With respect to Dr. Scoggin's studies of the cord, the greater movement of sugars out of the perfused structure, as compared with movement into it, might represent filtration in response to the hydrostatic pressure in the blood vessels, but other data show equal movements of water out of and into the cord. The perfusion medium, Locke's solution, has no oncotic pressure and the perfusion pressure, though not specified, must have been appreciable. I would expect the perfused cord to become edematous and lose water to the bath, but the calculated transfers do not support my guess.

DR. SCOGGIN (Closing). In response to Dr. Johnson's questions, there was a specific reason why D-histidine was not tested. In the studies done with the placental membrane by Plentl and others, they labeled their own D- and L-histidine. Unfortunately, I do not have the facilities to do this, and after starting on this series of experiments, it became apparent that labeled D-histidine was not commercially available. Therefore, I had to go around with all the other amino acids and simply test the available labeled L-histidine.

In response to Dr. Johnson's second question, there is evidence available that the membrane is alive and metabolically active at the completion of these experiments.[1–3] Basically, Brame and his colleagues measured oxygen consumption by the amnion both at the beginning and at the end of the experiments and found that the oxygen consumption was real and in the area of about 0.7, which is suggestive of viability. He did some other studies which I will mention in relation to Dr. Wynn's question.

Pressure differential certainly could be important. My personal feeling is that the design and the need for the perfusion of all three vessels is the major difference as pointed out by Dr. Wynn. When all three vessels were cannulated, there was not any real difference between transfer in one direction or the other of water. Measurements were taken in these latter experiments of perfusion pressures with perfusion between 225 and

250 ml. per minute through the three vessels simultaneously. The pressure within the vessels was approximately 10 to 12 mm. Hg. I would agree with Dr. Johnson that the increased transfer when all three vessels are perfused was due to the increased area and the increased amount of perfusate. Our measurement of area was the outside area of the cord. We were not able to measure the inside area or the surface area of the vessels themselves.

Dr. Wynn again raises the question of diffusion vs. exchange, and I would say with regard to his final remarks that our calculations were indeed made from the appearance of the tracer in the initially untagged compartment, as he suggested should have been done.

The presence of enzymes in the amnion is well established by Dr. Wynn in his studies. Also in 1970 Dr. Brame, in transfer studies, showed that there was indirect evidence for the presence of enzymes because enzyme poisons affected the consumption of oxygen, as might have been expected if enzymes were present.

The cord, finally, does not lose water, as Dr. Wynn thought it might do, but actually in fact picks up a little water during these experiments. Initial measurements were made after a period of equilibration because the cord actually increases in volume and weight by about 30 per cent during the experiments.

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Neurohormonal control of gonadotropic secretion in primates

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With immunohistochemical techniques, products from both the parvicellular system (such as Gn-RH) and from the magnocellular system (vasopressin, oxytocin, and neurophysins) have been shown to be present in the neurons ending around the hypothalamopituitary portal capillary system. A neurosurgical technique for prolonged collection of pituitary stalk blood has been developed. Gn-RH, vasopressin, and neurophysins have been found in large amounts in pituitary stalk blood. Gn-RH levels were found to vary in a pulsatile fashion, thereby providing direct evidence for hypothalamic modulation of the discharge of LH by the pituitary. The finding of large amounts of estrogen-neurophysin in portal vein blood adds further evidence to the hypothesis that "estrogen" neurophysin may play a role in the control by the hypothalamus over the secretion of gonadotropins.

ANATOMICALLY and physiologically, the female reproductive system is composed of three basic elements—the hypothalamus, the pituitary, and the ovaries. The pituitary controls the ovaries by the secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH); the ovaries regulate gonadotropic secretion through the important feedback role of the gonadal steroids. Communications between the pituitary and the ovaries, and reciprocally between the ovaries, the hypothalamus, and the pituitary, is via the peripheral circulation into which pituitary and gonadal hormones are secreted. The communication between the hypothalamus and the pituitary is, however, much more complex, and is illustrated in Fig. 1. As was shown initially by Scharrer and Scharrer,[1] the posterior pituitary (or pars nervosa) is a direct prolongation of the hypothalamus. Neurohormones (oxytocin, vasopressin, and related peptides such as the neurophysins) are transported directly to the posterior hypophysis within axons which originate from the supraoptic and paraventricular hypothalamic nuclei and are part of the magnocellular system. In contrast, neuronal pathways play only an unimportant role in the relationship between the hypothalamus and the anterior pituitary (or pars distalis); the hypothesis advanced by Harris[2]—that anterior pituitary secretion is controlled by neurohormones elaborated in the brain and transported to the pars distalis by vascular pathways—is now generally accepted. Most of the blood supplying the pars distalis is carried within the hypothalamopituitary stalk by long portal vessels originating from a primary capillary bed in the median eminence of the hypothalamus.[3] Other vessels, the short portal vessels, take their origin from a primary capillary bed situated in the lower infundibular stem. Most of the pituitary stalk blood has therefore transited through a region of the hypothalamus known to contain high concentrations of substances which have been shown to stimulate the secretion of hormones by the pituitary. Some of these neurohormones have recently been isolated in pure form and their structures identified. An example is the gonadotropin-releasing hormone (Gn-RH, LH-RH), a decapeptide isolated from sheep hypothalami.[4] Histochemical techniques using antibodies raised to synthetic Gn-RH have shown that this decapeptide, or a compound with identical immunologic properties, can be found in high concentrations around the portal capillaries in the median eminence.[5]

By means of similar immunohistochemical techniques, neurohormones such as neurophysins, oxytocin, and vasopressin can be traced along the entire

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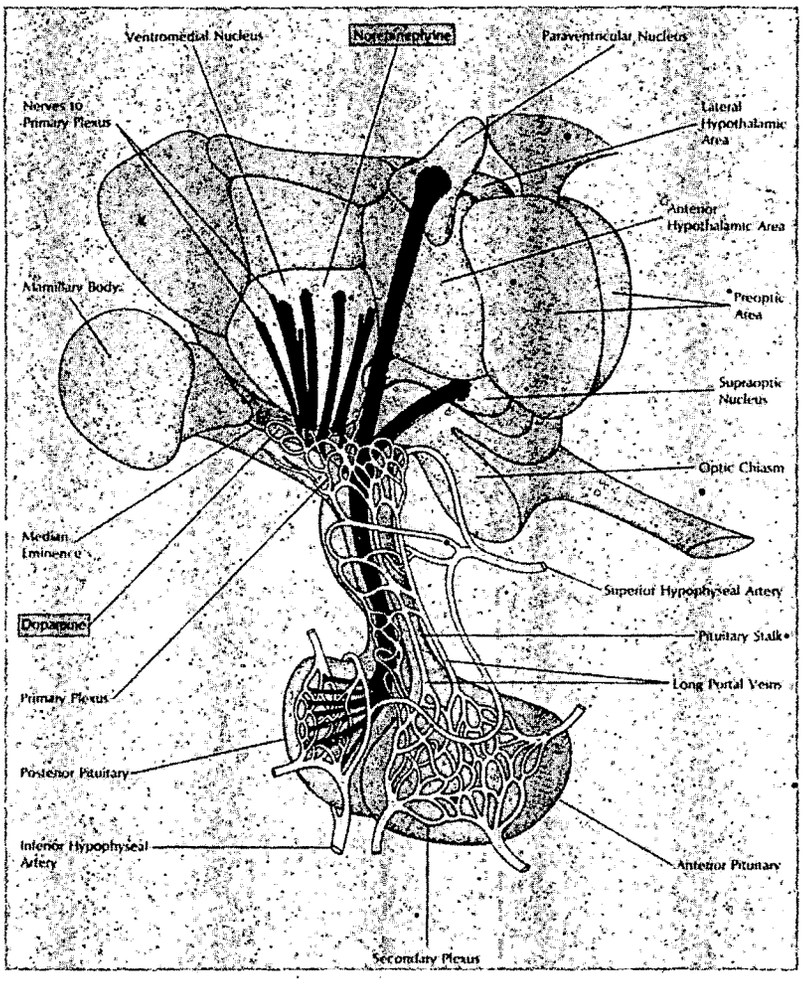


Fig. 1. Schematic diagram of the hypothalamopituitary axis. (Reprinted with permission from Frohman, L. A.: Hospital Practice 10: 54, 1975.)

hypothalamic magnocellular system and into the posterior pituitary. Recently, we made the surprising finding that these neurohormones, which were thought to be exclusively posterior hormones, also accumulated in the external layer of the median eminence around the portal vessels[6] (Fig. 2). This finding was of great interest because it indicated a possible alternate route of transport for hormones originating in the magnocellular system and suggested a possible direct role for these posterior pituitary hormones on the function of the anterior pituitary gland itself. Specifically, this finding provided an anatomical basis to explain reports in the literature[7] of a possible role of vasopressin on growth hormone and ACTH secretion or of oxytocin on reproductive function.

Recent studies from our laboratories have presented evidence for a role of neurophysins in reproductive processes. As in other endocrine areas, the sudden rapid progress in this field became possible as a result of the introduction of radioimmunoassay techniques which made it possible to detect neurophysins in small volumes of peripheral blood.[8] Radioimmunoassays have been developed for two separate neurophysins: one called "nicotine" neurophysin and the other called "estrogen" neurophysin. The administration of nicotine or experimentally induced hemorrhage increases the circulatory levels of "nicotine" neurophysin (without a concomitant change in the secretion of "estrogen" neurophysin); vice versa, estrogens will increase "estrogen" neurophysin in peripheral blood, while "nicotine" neurophysin levels remain unchanged. When neurophysins were assayed daily throughout the menstrual cycle, it was found that, at midcycle, the ovulatory surge of LH was invariably accompanied by a release of "estrogen" neurophysin.[9] Since it is well established that in primates, as well as in other species, the surgelike (midcycle ovulatory) secre-

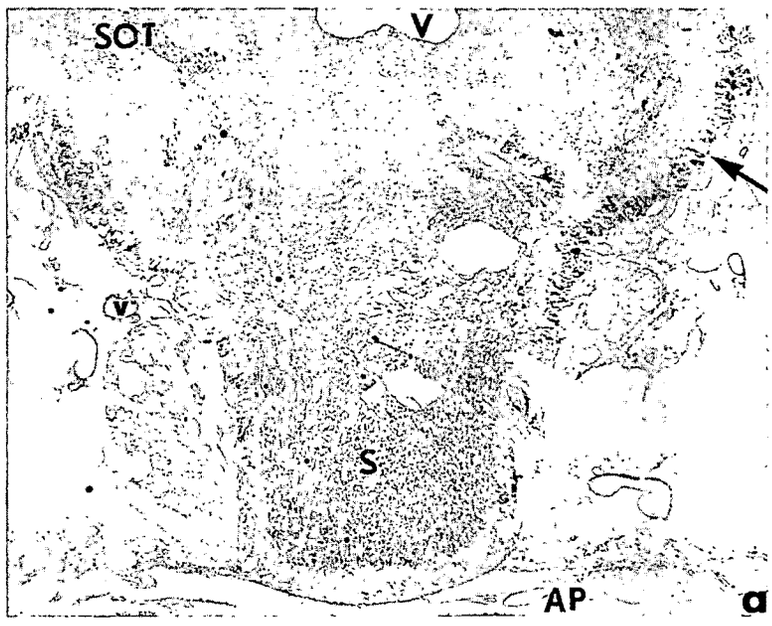


Fig. 2. Distribution of neurophysin in a coronal section (×43) of monkey hypothalamus at the level of the median eminence and pituitary stalk, by immunoperoxidase technique. Intense staining is seen in the external layer of the median eminence (arrow). *SOT;* Supraopticohypophyseal tract; *AP;* anterior pituitary, *V*, third ventricle; *v*, cross-section of a portal vessel.

tion of LH is controlled by estrogens,[10, 11] the finding of a midcycle peak of "estrogen" neurophysin raised the possibility that neurophysins may be related to the positive feedback effect of estrogens upon LH. Fig. 3 illustrates a study in which estrogens were administered to ovariectomized rhesus monkeys to evaluate the effect of such treatment upon the secretion of LH and "estrogen" neurophysin. The results clearly show that there is a close temporal correlation between the rise in LH and the rise in "estrogen" neurophysin, and that the latter one invariably precedes the LH surge by a few hours.

Although evidence at hand suggested that Gn-RH was, in the primate also, the neurohormone responsible for the release of FSH and LH, direct corroboration of this hypothesis could be obtained only by demonstration of significant amounts of Gn-RH in pituitary portal blood. Similarly, before we could assign a role to oxytocin, vasopressin, or neurophysin in the control of anterior pituitary secretion, it was necessary to demonstrate that these substances were present in large amounts in pituitary stalk portal blood. The study of the contents of portal vein blood was obviously an important key to our understanding of hypothalamic control of the pituitary and therefore we embarked on an attempt to collect pituitary stalk portal blood in rhesus monkeys.

The surgical approach to the pituitary stalk in rhesus monkeys presents certain difficulties not seen in rodents. Indeed, the pituitary gland is almost entirely encased in the bony sella and this bone is quite thick. In our studies, we approach the pituitary stalk via the transorbital route, a route which has the advantage of not disturbing either the brain or the stalk during the procedure. In the first step, the globe, muscles, and retro-orbital fat are removed from the orbital fossa. After drilling through the bone, part of the chiasm is resected, the ophthalmic artery is cauterized and sectioned, and the dura is exposed and cut. At this point the entire pituitary stalk can be visualized, including the long portal vessels on its surface.

In a first series of experiments, pituitary stalk portal blood was collected with microcannulas introduced into single-portal vessels. The technical difficulties involved in such a cannulation can be appreciated if consideration is given to the fact that the diameters of these vessels range from 40 to 60 micrometers. Analysis of the collected samples revealed several important and some quite surprising findings. Significant levels of Gn-RH were found and the hormone in portal blood was immunologically indistinguishable from synthetic Gn-RH. Both "estrogen" and "nicotine" neurophysins were detected. Their levels in portal blood were higher than those observed in simultaneously collected peripheral bloods (Table I).[8] These findings confirm our initial hypothesis that neurophy-

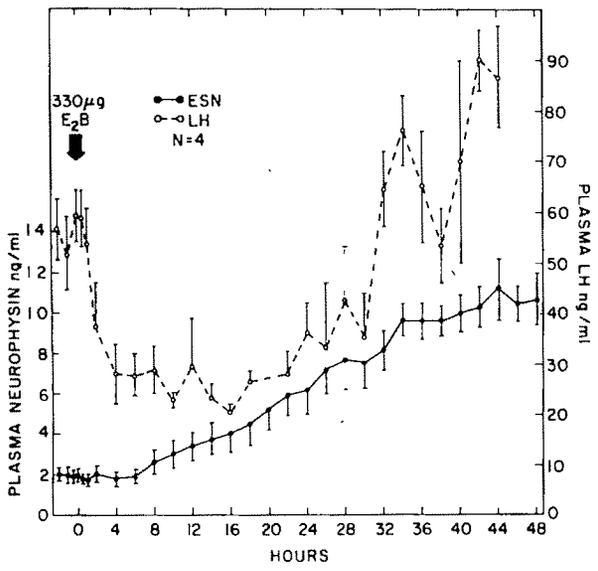


Fig. 3. LH and "estrogen" neurophysin *(ESN)* secretion following injection of estradiol benzoate *(E_2B)* to ovariectomized rhesus monkeys.

Table I. "Estrogen" neurophysin, "nicotine" neurophysin, vasopressin, and gonadotropin-releasing hormone measured by radioimmunoassay in simultaneous samples of rhesus monkey systemic venous blood and of pituitary stalk blood (from single portal vessels for ESN, NSN, and VP, from whole stalk blood for GN-RH)*

	ES-NP (ng./ml.)		*NS-NP (ng./ml.)*		*VP (pg./ml.)*		*Gn-RH (pg./ml.)*	
Monkey	*PB*	*SB*	*PB*	*SB*	*PB*	*SB*	*PB*	*SB*
557	1.4	124	4.4	100	—	—	—	—
561	3.0	25	2.0	10	35	11,000	—	—
564	4.6	240	2.1	88	50	16,000	—	—
448	2.2	28	2.0	48	40	14,500	—	—
607							U	77
596							U	74
603							U	61
572							U	40

*Abbreviations: ESN, estrogen neurophysin; NSN, nicotine neurophysin; VP, vasopressin; Gn-RH, gonadotropin-releasing hormone; U, undetected.

sins are secreted into pituitary portal vessels and that they, therefore, may play a role in the control of anterior pituitary secretion. The exact nature of this role remains to be determined. Of particular significance were the high levels of vasopressin found in pituitary stalk blood, since these findings revived the possibility, suggested several years ago, that vasopressin may act as an ACTH-releasing hormone. Initial evidence in favor of this hypothesis was derived from experiments in which ACTH release was observed following the systemic administration of large amounts of vasopressin.[7] However, doubts had been expressed about the physiologic significance of these experiments. It was also argued that in physiologic and pathologic circumstances, the increased secretion of ACTH was not accompanied by any of the other known biologic effects of vasopressin, which were to be expected if vasopressin were the factor responsible for ACTH release by the pituitary. These criticisms do not apply to our observations. Vasopressin in the pituitary portal vein blood reaches the pituitary without further dilution or metabolism and the concentrations observed by us could be sufficient to produce ACTH release by the pituitary, while insufficient to produce systemic effects. Further studies will be necessary to establish without doubt the role of vasopressin as an ACTH-releasing factor.

Although these experiments clarified certain aspects of the control of the hypothalamus over the pituitary, the approach used did not allow us to collect blood for prolonged periods of time and in this way to determine whether Gn-RH was secreted at a constant rate or in a pulsatile pattern. This question was of importance since it is known that tonic secretion of LH and FSH represents the integration of episodic pituitary discharges.[12] This episodic pattern of secretion has been observed in all animals that were studied and is most pronounced in castrated animals.[13] In oophorectomized rhesus monkeys, the frequency of pulsatile LH release varies from 1 to 3 hours, as is shown in Fig. 4. Since the cannulation of single-portal vessels would not permit us to collect blood for significant periods of time, our experimental approach was modified. After heparinization of the animal, the stalk was cut at the level of the diaphragm sellae with microscissors, and a micromanipulator was used to fit a collector over the stalk. The collector was then connected to an exfusion pump which was set at a rate of 25 to 40 microliters per minute. An air-embolator technique[14] was used to separate segments of blood, thus preventing mixing. With this technique, pituitary stalk blood samples were collected continuously for periods of up to 8 hours. During the collection of blood, the animals were sedated with phenobarbital or sernylan and in earlier experiments we had shown that this treatment does not interfere with the pulsatile release of LH in ovariectomized monkeys.[15] The results of these experiments indicate that Gn-RH release into pituitary stalk portal blood is clearly pulsatile in nature.[16] An example of a 5 hour portal blood collection in an ovariectomized

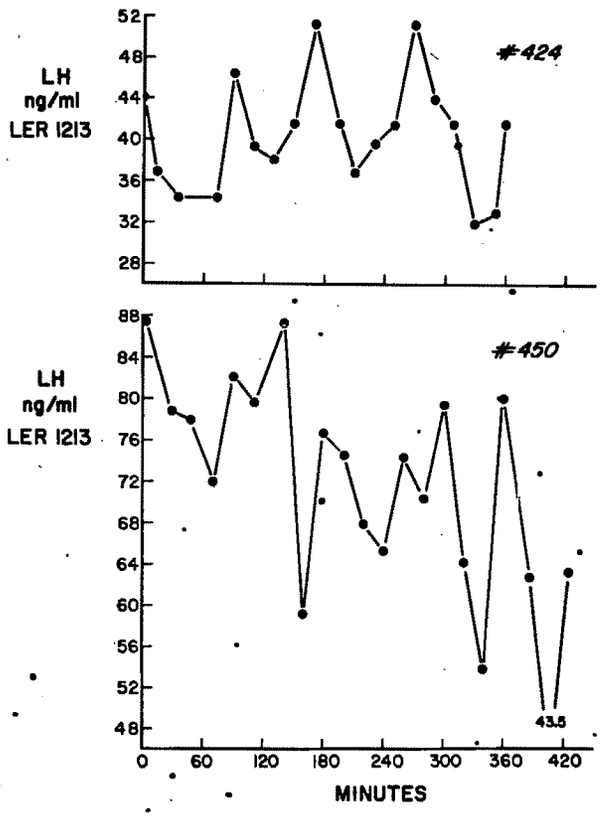


Fig. 4. Pulsatile LH release in two ovariectomized rhesus monkeys.

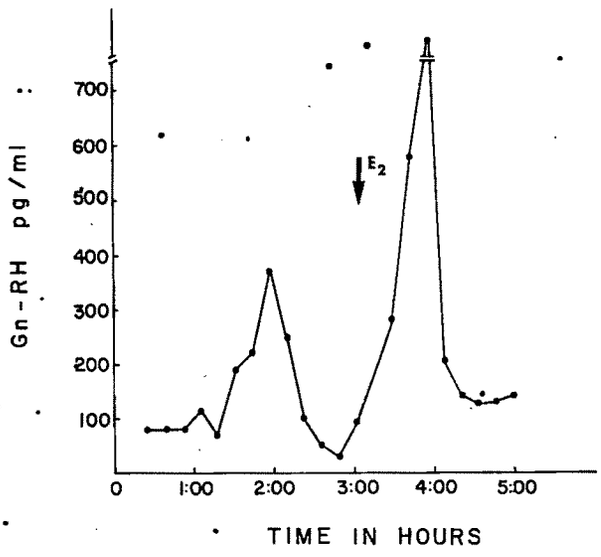


Fig. 5. Results from a 5 hour pituitary portal blood collection in an ovariectomized rhesus monkey. Gn-RH levels were measured by radioimmunoassay. The arrows indicate the time of injection of sernylan and pentobarbital. E_2, Estradiol-17β, 1,000 ng.

monkey is illustrated in Fig. 5. Unfortunately, the section of the pituitary stalk that is necessary for the blood collection procedure does not allow a direct comparison of the Gn-RH pulses in portal vein blood and the pulses of LH in peripheral blood. However, the results indicate that the time intervals between Gn-RH pulses in pituitary stalk blood are within the range of those observed for LH in ovariectomized monkeys. In a series of experiments to be reported, a systemic dose of estradiol was given during continuous collection of pituitary portal blood. This dose simulates estrogen levels seen during the early follicular phase and has been shown to depress circulating levels of LH in the rhesus monkey.[17] LH decrease is rapid and occurs within minutes after the administration of estradiol. Surprisingly enough, in our experiments, the administration of estradiol did not produce a decrease of Gn-RH in pituitary stalk blood (Fig. 5). These unexpected results are certainly intriguing. Similar types of results were reported by Seyer and Reichlin[18] in man, using a bioassay for Gn-RH in peripheral blood. If these observations are confirmed, it may be concluded that estradiol exerts negative feedback effects directly on the pituitary gland, thereby rendering the hypophysis unresponsive to Gn-RH stimulation.

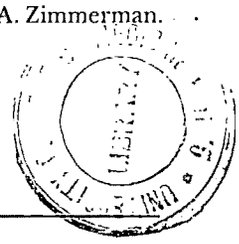
Much remains to be learned about neuroendocrinology of reproduction, but patterns of controls start to emerge. The hypothalamus controls pituitary FSH and LH secretion through the neurosecretion of Gn-RH (LH-RH) which reaches the pituitary via the long portal veins of the pituitary stalk. It remains uncertain whether there are hypothalamic factors other than Gn-RH stimulating the synthesis and release of FSH and LH. Current facts are consistent with the theory that there is only a single factor, Gn-RH, responsible for the control of both FSH and LH. The control of gonadotropin secretion by Gn-RH is not a simple one and there is ample evidence that the response of the pituitary to Gn-RH is modulated by a number of other factors, including the effect of estrogens upon pituitary sensitivity. This complexity of the pituitary response greatly limits the usefulness of the administration of Gn-RH as a test for pituitary function. Our finding of a pulsatile secretion of Gn-RH into the portal vein system makes it likely that the pulsatile secretion of FSH and LH results from a pulsatile input from the hypothalamus.

The role of neurophysins in the control of pituitary function remains to be clarified. Our finding of large amounts of "estrogen" neurophysin in pituitary portal blood, together with the cyclic changes in the levels of

"estrogen" neurophysin during the menstrual cycle, makes it likely that "estrogen" neurphysin plays a role in the positive feedback of estrogens upon LH release.

It must be noted that "estrogen" and "nicotine" neurophysins are the only hypothalamic neurohormones that can be conveniently measured in the peripheral circulation. The possibility of using "estrogen" neurophysin as an index for hypothalamic function and as a method to classify various forms of ovarian failures is now being explored in our laboratory.

The work reported in this paper is based upon the studies of my many collaborators in the neuroendocrine unit of the Department of Obstetrics and Gynecology in the College of Physicians and Surgeons, Columbia University. Special acknowledgement is made to the contributions of Drs. S. Araki, P. W. Carmel, I. Dyrenfurth, M. Ferin, A. Frantz, M. K. Husain, R. Jewelewicz, M. Warren, and E. A. Zimmerman.



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Discussion

DR. JAMES WARREN, St. Louis, Missouri. This elegant piece of work demonstrates that monkey pituitary portal blood can be collected and that its content of vasopressin, neurophysin, and GnRh can be quantitated. This is extremely important because attempts to quantitate endogenous GnRH in peripheral blood have failed. The work presents several distinct observations. (1) Vasopressin concentrations in pituitary portal plasma are 300 times peripheral levels, reviving the possibility that it may serve a "releasing hormone" role. (2) GnRH concentration displays "pulse" at 2 to 3 hour intervals which may cause the LH pulses previously observed and described. (3) after ovariectomy, baseline levels of GnRH are elevated twofold and pulses are strikingly higher. (4) Doses of estradiol-17β which suppress LH do not suppress GnRH, leading Dr. Vande Wiele to suggest that the steroid enacts its negative feedback directly on the pituitary gland.

More indirect studies, done in the past, relating to estrogens, exogenous GnRH administration, and LH response have sometimes been confusing and subject to subsequent revision. Originally estrogen seemed only to suppress LH release. Later, using estradiol benzoate, Knobil was able to show an early suppression and later an increase in plasma LH in the monkey. Radioimmunoassay methods for GnRH in peripheral blood have been devised but never validated in terms of endogenous levels and, indeed, are held by most to be worthless. In terms of estrogen effects on pituitary sensitivity to GnRH, Barraclough has added estradiol to a superfusion system of rat pituitary quarters and reported enhanced LH release after addition of GnRH. Further, Allen, Mahesh, and co-workers, using the rat pituitary isolated in situ by stalk section and plate insertion, have reported that daily estrogen administration enhances LH response to exogenous GnRH. While these studies would appear to contradict Vande Wiele's fourth observation, they are done in the rat and the second is a chronic experiment. Data in the

primate capable of bringing real understanding of the interrelationships of estrogen, GnRH, and LH release will be fitted in place only when minute amounts of estrogen can be placed stereotaxically in specific areas of the central nervous system (especially hypothalamus and pituitary) and the effects on GnRH in pituitary portal plasma and LH levels in jugular or other peripheral plasma of intact animals studied over reasonably prolonged periods. I suspect this is Vande Wiele's ultimate goal and I would ask him if there are any further observations at this time.

In passing I would mention that we have (as a prerequisite to efforts to characterize GnRH receptors with photoaffinity labeling GnRH derivatives) studied the binding of ^{125}I-GnRH to bovine pituitary cell membranes. In the steer there appear to be two GnRH-binding sites with dissociation constants of $0.8 \times 10\text{-}^{10}M^{-1}$ and $8 \times 10^{-10}M^{-1}$ or a cooperative system with binding in these ranges. Although the species are different, it is interesting that Vande Wiele's physiologic GnRh surges ranging up to approximately 10^{-10}M and 7×10^{-10}M in the monkey with and without ovaries, respectively, would be pertinent to filling these sites.

DR. BRIAN LITTLE, Cleveland Heights, Ohio. I would like to ask two or three questions for my own education and perhaps that of some of the rest of us.

One is, what is neurophysin and is it specific that one is estrogen dependent and the other is nicotine sensitive, or is this just artifact? In other words, is there any direct correlation between the estrogen sensitive neurophysin and LH for example.

You talked about negative feedback to estrogen; was there any positive feedback in response to the estrogen that you administered?

And, finally, the selective distribution of estradiol throughout the brain in the rat and in the monkey demonstrates a high concentration of estradiol associated with the pituitary. There is a much lower selective concentration of estradiol in the region of the hypothalamus. There is the possibility that the estradiol is acting on the pituitary, but why is the selective distribution of estradiol so minimal in the hypothalamus in comparison, for example, to the cerebral cortex?

DR. R. A. MUNSICK, Indianapolis, Indiana. How accurate is your assay system for vasopressin? Does it differentiate between arginine and lysine vasopressins? Do you happen to know if it will tell the difference between arginine vasopressin and arginine vasotocin, and did you measure oxytocin? What exactly was your assay system?

DR. WILLIAM N. SPELLACY, Gainesville, Florida. Currently, most investigators use 100,000 picogram injections of Gn-RF to look for separation of reproductive dysfunctional states. You report peak levels of 400 picograms in the hypophyseal stalk blood. Do you think that we are using too high a dose of Gn-RF in the clinical hypothalamic-pituitary studies to be able to accurately separate dysfunctions of the hypothalamic area?

DR. VANDE WIELE (Closing). It is correct that the frequency of the Gn-RH pulses in the portal vein blood does not fit with what we know about the frequency of the pulses of FSH and LH in peripheral blood. We have more data than what I have shown; we found the same discrepancy in the additional data and therefore this is likely to be real. Actually, one should not expect a perfect fit between these frequencies. Indeed, a similar discrepancy has been found for the frequencies of the pulses of ACTH and cortisol, and of LH and testosterone. There is ample evidence that the sensitivity of the pituitary to stimuli changes with time, and therefore one could predict that the frequency of the Gn-RH and FSH-LH pulses would not coincide.

I may have confused you about the experiments in which we administered estradiol while collecting portal vein blood. The conclusion we drew from these experiments was that the mechanism by which estradiol inhibits LH secretion is not by decreasing the secretion of Gn-RH, but, more likely, by making the pituitary less sensitive to the Gn-RH stimulus. There is other experimental evidence indicating that this is the mechanism.

We have tried to study the feedback effect of estradiol upon FSH-LH secretion by implanting estradiol directly into the hypothalamus. You are obviously familiar with all the vagaries of this approach, which has often been discussed by Bogdanove. There is always the possibility that what we implant in the hypothalamus reaches the pituitary and vice versa, and therefore we have recently abandoned this approach.

Nicotine and estrogen neurophysin can be separated by chemical methods. There is virtually no crossreaction in our radioimmunoassay system between nicotine and estrogen neurophysin. This can be clearly demonstrated by experiments in which nicotine or estrogens are administered and both neurophysins are measured simultaneously. In experiments in which nicotine is administered, there is an increase in the nicotine (but not in the estrogen) neurophysin. The opposite is found in experiments in which we administered estrogens.

I am interested in Dr. Little's findings about the receptors for estradiol in the pituitary. We feel that the pituitary plays a much larger role in estrogen feedback than was previously realized and your findings are further support for our conclusions.

The assay we use for vasopressin is a radioimmunoassay developed by Dr. Hussain at the College of Physicians and Surgeons. I must admit that I do not know whether the assay would measure both arginine and lysine vasopressin but I would guess it does.

I agree that the amounts of Gn-RH administered

to test the responsiveness of the pituitary are unphysiologic. There is, however, no way around this unless one is able to administer the Gn-RH directly into the portal veins. This has actually been done by J. Porter who showed that by this route very small amounts of Gn-RH will stimulate the secretion of FSH and LH. In most experimental situations, however, this route of administration is impractical or impossible. If we administer Gn-RH into a peripheral vein, we have to administer much larger amounts since by this way only a very small amount of Gn-RH will reach the portal vein system. This would be true with any compound but even more so with Gn-RH since the half-life of disappearance from the blood of Gn-RH is measured in minutes.

Rate of transport of radioactive ovum models through the oviducts of individual rabbits

CARL J. PAUERSTEIN, M.D.
BARRIE J. HODGSON, PH.D.
San Antonio, Texas

The transport of radioactive ovum models of 100, 200, 400, 800, and 1,000μ diameter through the oviducts was measured. Four surrogates of a given size were transferred into each oviduct 6 hours after injection of human chorionic gonadotropin (HCG). The surrogates were located in the oviducts 24, 48, 60, 66, and 72 hours after HCG injection with a collimated end-window Geiger-Mueller tube. Thus the time-course of the transport of each surrogate, from ampulla to uterus in the same animal, was plotted. The time-course of surrogate transport was directly related to surrogate size. Our data suggest that normal tubal patency and ovum transport function can be tested by introducing radioactive ovum surrogates of appropriate size into the oviducts and monitoring their time of arrival at the uterus.

A TUBAL FACTOR is involved in about one third of infertile marriages. Despite the elapse of 60-odd years since Cary's[1] use of Collargol to visualize the Fallopian tubes by radiography, and of 55 years since Rubin's[6] preliminary report of the use of transuterine gas insufflation for the assessment of tubal patency, no major advances have been made in the clinical measurement of tubal function. Procedures in current use allow evaluation of the patency and external appearance of the Fallopian tubes, but not of their physiologic functions.

For the past several years, we have been studying ovum transport in the rabbit oviduct, and have evolved an admittedly oversimplified hypothetical model of the regulation of the rate of transport, which postulates that contractions of the myosalpinx are the major force driving ovum transport, and that transport rates are the resultant of this force working against opposing resistances.[4] In support of our efforts to define the location and nature of these resistances, our laboratories manufactured a plastic ovum surrogate made sufficiently radioactive with ^{125}I to be detectable through the wall of the intact oviduct.[2] We then studied the transport of these plastic microspheres through the oviducts of rabbits killed in groups at various intervals after the injection of human chorionic gonadotropin (HCG).[3, 5] These experiments revealed that the diameter of the microspheres is an important determinant of transport rate, and suggested that the rate at which ovum models of appropriate size were transported might provide a useful index of tubal function. This premise could not be properly tested by reconstructing transport rates from animals killed at intervals, but rather required tracking the transport of ovum models from ampulla to uterus in the same animal. This report

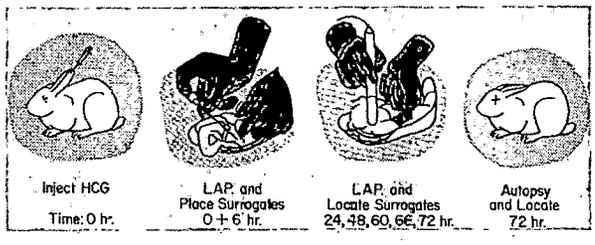


Fig. 1. Design of experiment (see text for explanation).

From the Center for Research and Training in Reproductive Biology and Voluntary Regulation of Fertility, The University of Texas Health Science Center at San Antonio, Department of Obstetrics and Gynecology.

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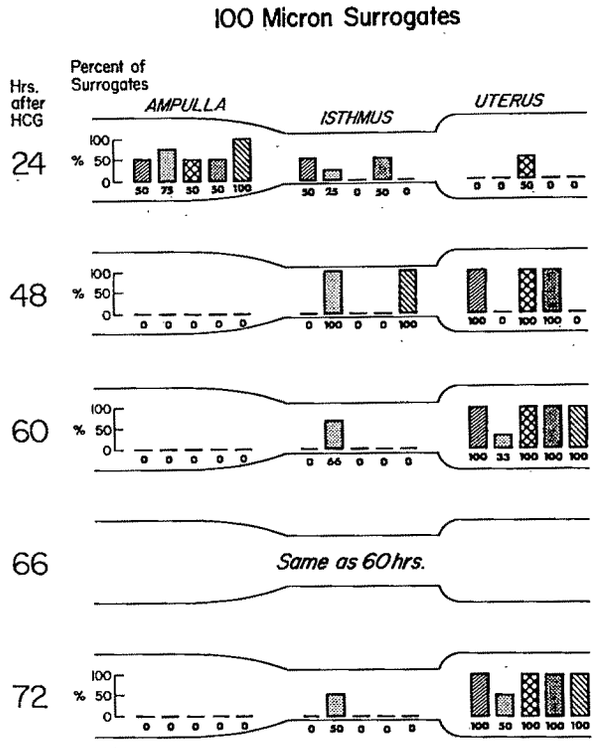


Fig. 2. The per cent of 100μ spheres located in the ampulla, isthmus, and uterus are plotted for each time interval. Each bar represents an individual rabbit. For example, in the first rabbit of this group, 50 per cent of the surrogates were in the ampulla and 50 per cent were in the isthmus at 24 hours; 100 per cent were in the uterus at 48 hours.

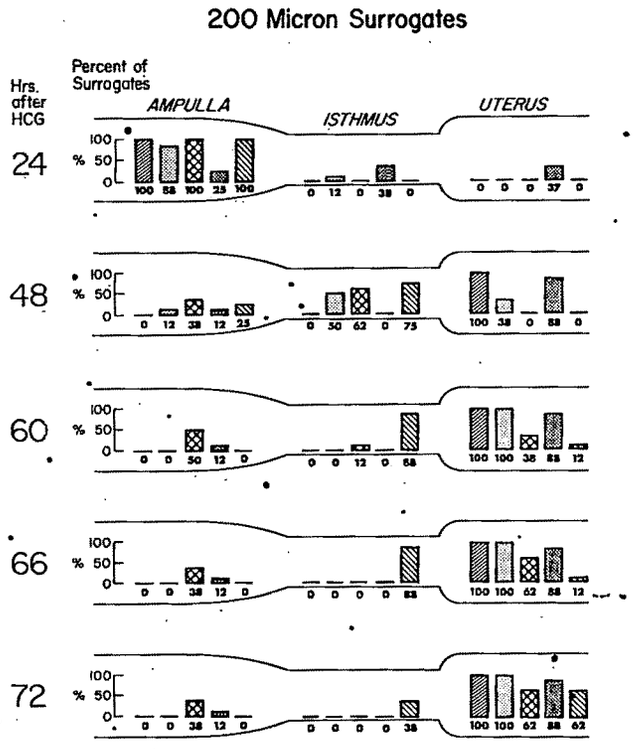


Fig. 3. Same conventions used as in Fig. 2.

describes the results of such experiments and discusses the physiologic and clinical implications of the data obtained.

Materials and methods

New Zealand white virgin does isolated for at least 30 days in a controlled environment were used in these experiments. The animals were divided into eight groups, each containing five animals. All animals were given 100 units of HCG injected into a marginal ear vein. Animals in Groups 7 and 8 were given an intramuscular injection of 250 μg of estradiol cyclopentylpropionate at the same time. Six hours later the oviducts were exposed through a vertical midline incision, under sodium pentobarbital anesthesia. The tip of a microsyringe was introduced 1 to 2 cm. into the ampulla of each oviduct via the abdominal ostium. Four radioactive plastic ovum surrogates, each containing 4 to 6 μCi of ^{125}I were introduced into each oviduct in 1 μl of tissue culture fluid (Fig. 1). The diameters of the microspheres tested were: Group I, 100 μ; Group II, 200 μ; Group III, 400 μ; Group IV, 600 μ; Group V, 800 μ; Group VI, 1,000 μ; Group VII, 200 μ, estrogen treated; Group VIII, 600 μ, estrogen treated.

Serial laparotomies were performed on each animal 24, 48, 60, 66, and 72 hours after the HCG injection. At laparotomy, the genital tracts were scanned with a collimated mica end-window Geiger tube, and the surrogates were located to within ± 1 min. The animals were killed at the 72 hour laparotomy by an overdose of sodium pentobarbital, the genital tracts were excised en bloc and trimmed and straightened, and the final position of each surrogate in the excised tracts was recorded.

The results were expressed in three ways. (1) The location of the surrogates in each animal was plotted as per cent of surrogates found in ampulla, isthmus, and uterus at each laparotomy. (2) A composite plot of the time-related location of the surrogates was constructed by treating the five animals of each group as one, and plotting the per cent of surrogates in each location at each time interval for the entire group. (3) Finally, the results were expressed as the time of initial entry of surrogates of a given size into the uterus, the time of entry of surrogates into the uterus of at least 60 per cent of the animals in each group, and the time of entry of 60 per cent of the total number of transferred surrogates into the uterus.

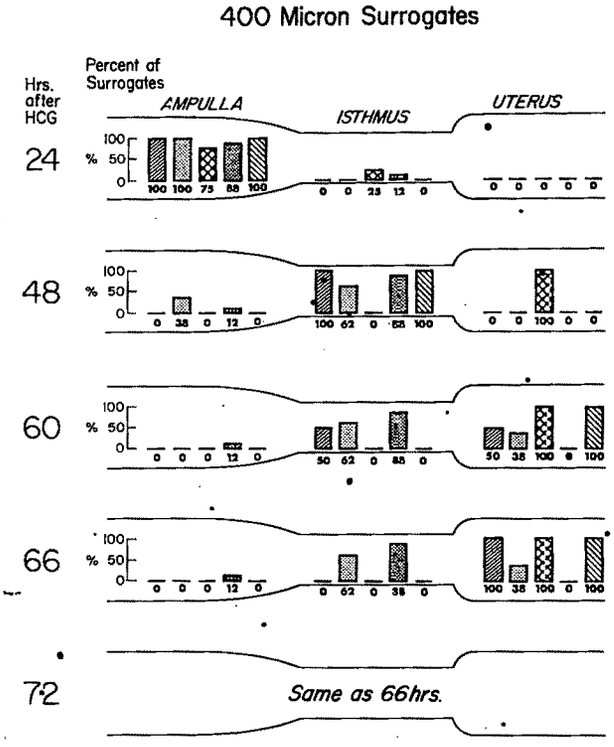


Fig. 4. Same conventions used as in Fig. 2.

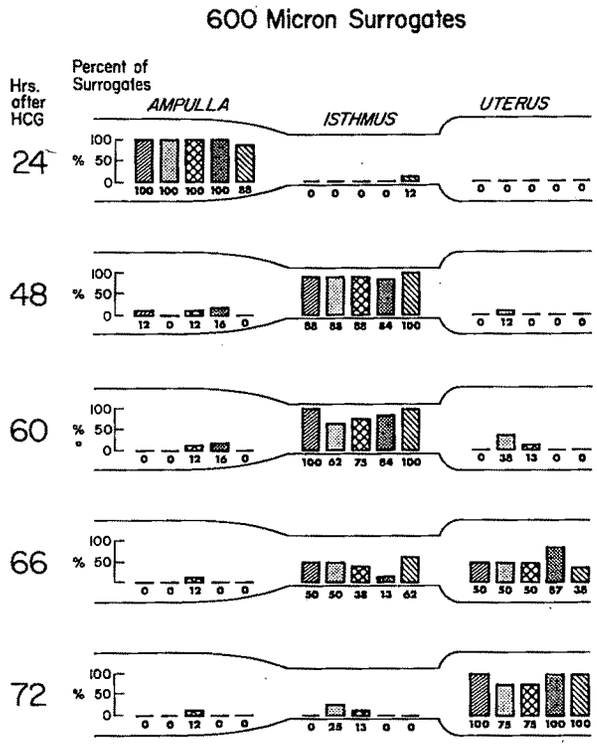


Fig. 5. Same conventions used as in Fig. 2.

Results

Location of surrogates in individual animals.

Group I—100 μ surrogates (Fig. 2). By 24 hours after HCG injection, surrogates had passed beyond the ampullary-isthmic junction (AIJ) in four of the five animals and had entered the uterus in one of the four. At 48 hours, all 100 μ surrogates were beyond the AIJ and had reached the uterus in three of five animals. By 60 hours, 100 μ spheres had reached the uterus in all animals.

Group II—200 μ surrogates (Fig. 3). By 24 hours after HCG, 200 μ spheres had traversed the AIJ in two of five animals and had entered the uterus in one. By 48 hours, 200 μ surrogates had passed beyond the AIJ in all animals and had reached the uterus in three. Surrogates had reached the uterus of all five animals by 60 hours after HCG.

Group III—400 μ surrogates (Fig. 4). Although 400 μ surrogates had negotiated the AIJ in two animals at 24 hours, and in all by 48 hours, uterine entry occurred in only one rabbit by 48 hours. By 60 hours, they had entered the uterus in four rabbits.

Group IV—600 μ surrogates (Fig. 5). The 600 μ microspheres were beyond the AIJ in only one animal at 24 hours but were in the isthmus of all animals by 48 hours. Although these surrogates reached the uterus in one animal by 48 hours, uterine entry in the majority of rabbits did not occur until 66 hours. The 600 μ ovum surrogates perfectly imitated the time course of normal ovum transport in the rabbit[4] even though they are about twice as large as the natural ovum with its mucin layer.

Group V—800 μ surrogates (Fig. 6). The 800 μ spheres traversed the AIJ in four of five animals by 48 hours. Surrogates did not reach the uterus in any rabbit until 66 hours, and a majority of 800 μ spheres never entered the uterus.

Group VI—1,000 μ surrogates (Fig. 7). Some 1,000 μ spheres passed beyond the AIJ in two animals at 48 hours and in three animals by 72 hours. None of these spheres reached the uterus by 72 hours.

Group VII—200 μ surrogates plus estradiol treatment (Fig. 8). In this group, estradiol prolonged the delay at the AIJ so that all microspheres were still in the ampula in three of five animals at 48 hours. Except for a few spheres in one animal at 72 hours, however, microspheres were never found in the isthmus. Any that passed the AIJ were found in the uterus.

Group VIII—600 μ surrogates plus estradiol treatment (Fig. 9). No surrogate passed beyond the AIJ in the group.

Composite time-course of surrogate transport.

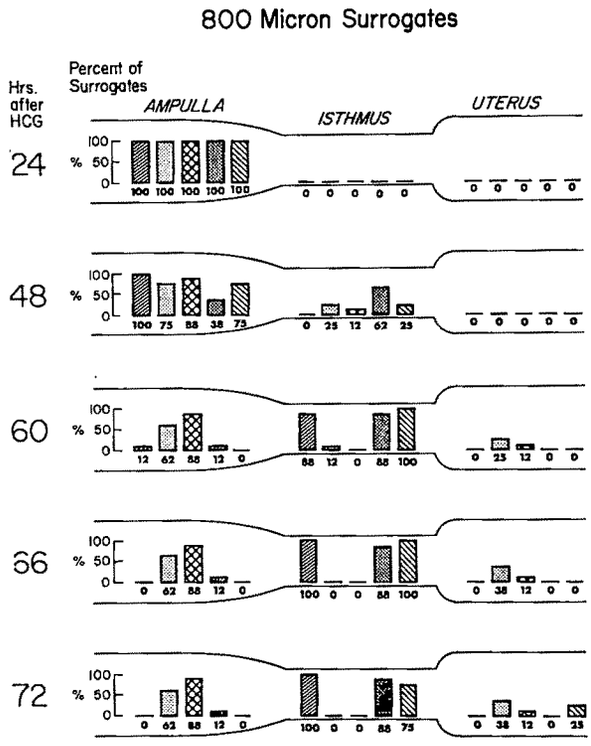


Fig. 6. Same conventions used as in Fig. 2.

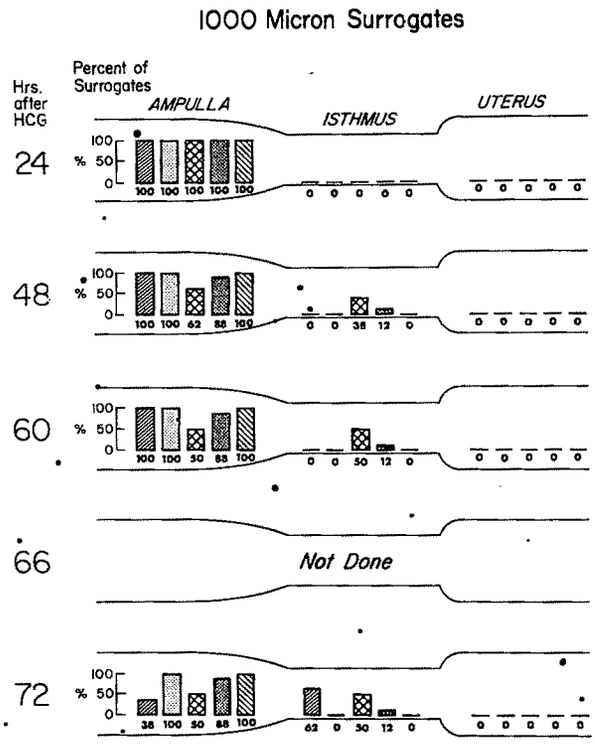


Fig. 7. Same conventions used as in Fig. 2.

Table I. Relationship of surrogate size to time of entry into uterus

Surrogate size (μ)	*Time of initial entry (hr.)*	*Time of entry in 60% of animals (hr.)*	*Time of entry of 60% of surrogates (hr.)*
100	24	48	48
200	24	48	60
400	48	60	66
600	48	66	72
800	60	72	*
1000	*	*	*

*Had not occurred at 72 hours.

After averaging the data for the five animals of each group, trends became apparent. The surrogates fell into three classes: (1) 100 and 200 μ, (2) 400 and 600 μ, and (3) 800 and 1,000 μ. Composite data for representatives of each of these classes appear in Fig. 10. Thirty-five per cent of 100 μ surrogates traversed the AIJ 24 hours after HCG. One third of these were in the uterus. By 48 hours, all 100 μ spheres were beyond the AIJ and 60 per cent had reached the uterus. Thus, a maximum of 40 per cent of these microspheres accumulated in the isthmus at any time.

In contrast, although 92 per cent of 600 μ surrogates were beyond the ampulla by 48 hours, only 2 per cent of them reached the uterus. The other 90 per cent were retained in the isthmus. Isthmic accumulation diminished markedly at 66 hours, at which time more than 50 per cent of 600 μ spheres were in the uterus.

Surrogates of 1,000 μ diameter were retained in the ampulla. Only 25 per cent were in the isthmus by 72 hours. None entered the uterus during the 72 hour interval studied.

Relationship of surrogate size to time of entry into uterus. The time of initial entry of surrogates into the uterus was directly size related (Table I). Except for 100 μ and 1,000 μ surrogates, some surrogates reached the uterus in each animal sooner than a majority of the total number of transferred surrogates reached the uterus in the entire group. For example, only 15 per cent of 800 μ surrogates reached the uterus, but three of the five rabbits contributed to this 15 per cent. The

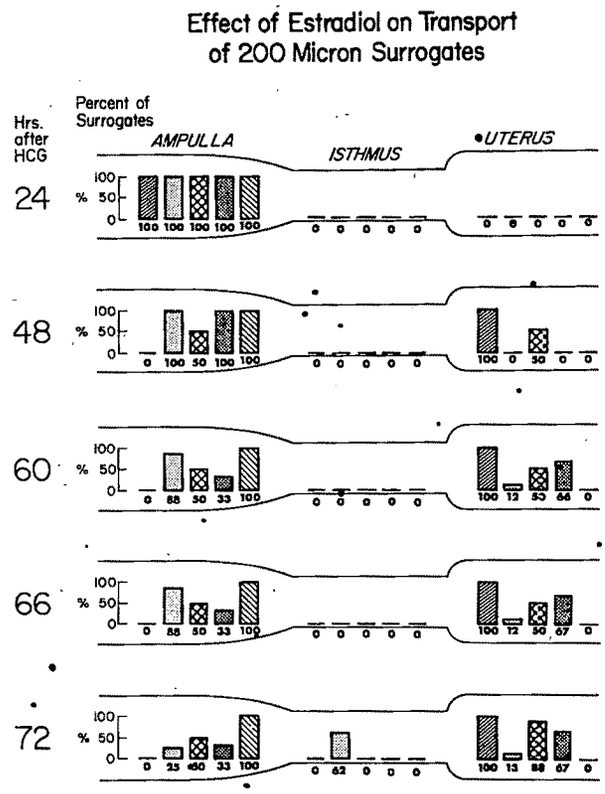


Fig. 8. Same conventions used as in Fig. 2.

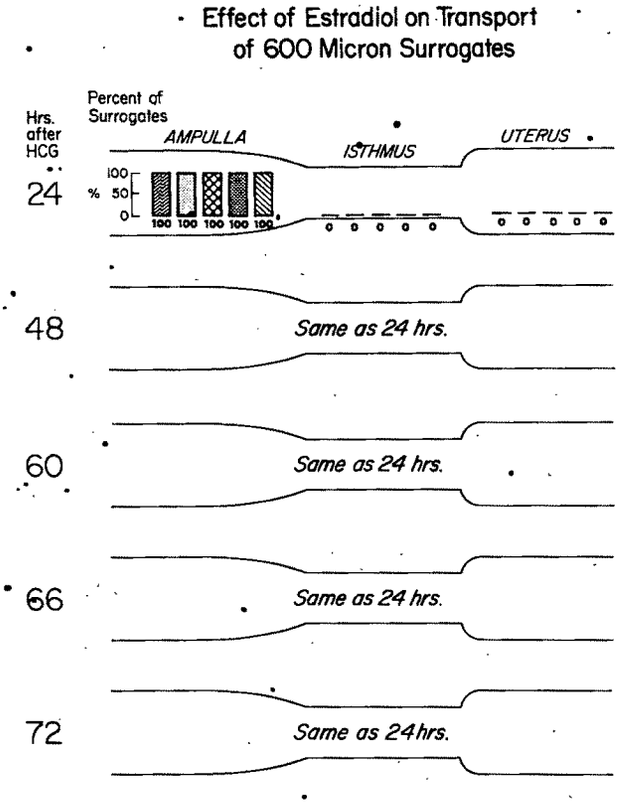


Fig. 9. Same conventions used as in Fig. 2.

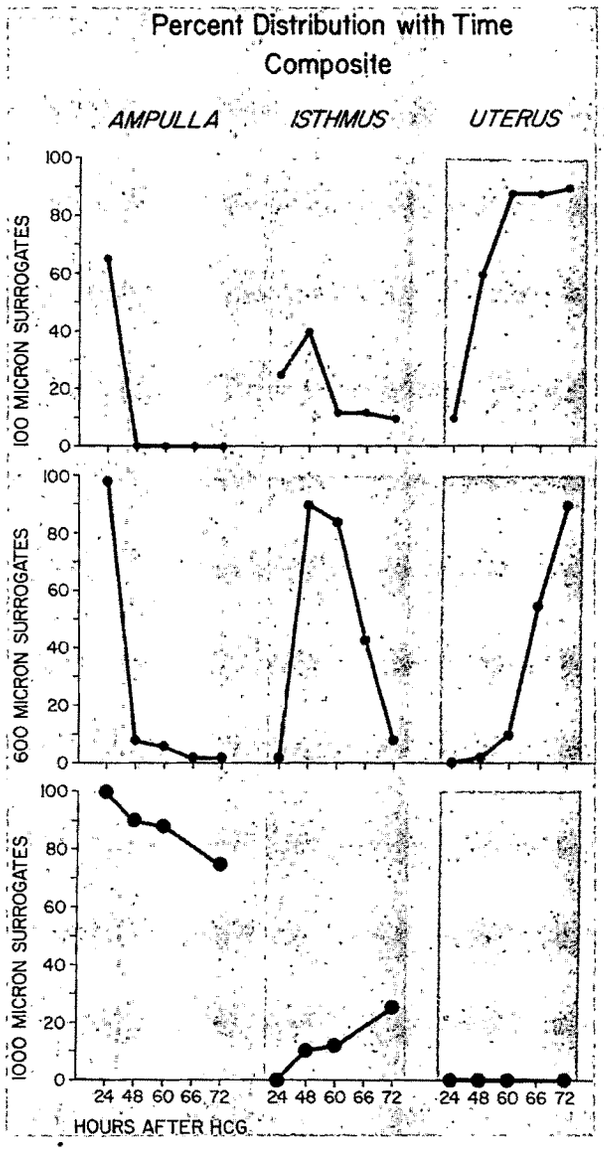


Fig. 10. Composite per cent distribution for the five animals in each of the 100, 600, and 1000μ surrogate groups is plotted. The percentages of surrogates in each location are shown on the vertical axis, and the time after HCG on the horizontal axis. For example, at 24 hours, 65 per cent of 100μ surrogates were in the ampulla, 25 per cent were in the isthmus, and 10 per cent in the uterus. Corresponding figures at 48 hours were 0, 40, and 60 per cent.

1,000 μ spheres never reached the uterus in any animal.

Comment

Surrogates of 100 to 200 μ diameter entered the isthmus and uterus by 24 hours after HCG. In contrast, 400 to 600 μ spheres did not enter the isthmus in large numbers until 48 hours, and significant entry to the uterus did not begin until 60 to 66 hours. Thus, 24

hours after HCG the AIJ restricts the passage of spheres 400 μ in diameter, but does not efficiently discriminate against those of 200 μ or less. Similarly, the isthmus offers resistance to the passage of particles 400 μ or larger, but not to those 200 μ or less. By 48 hours, either isthmic resistance is diminished or the propulsive forces begin to overcome it. The fact that 600 μ surrogates are retained in the isthmus until 66 hours, along with prolonged retention of 800 and 1,000 μ surrogates, favors decreasing resistance rather than increased propulsive forces, although both may be operative.

The effects of estradiol are particularly interesting. No 600 μ surrogate progressed beyond the ampulla of animals treated with estrogen. In contrast, although estrogen also caused some ampullary retention of 200 micron spheres, about one third of the 200 μ surrogates had traversed the AIJ by 48 hours and all of them were found in the uterus. We were fortunate enough to observe some surrogates traversing the entire isthmus in minutes after passing through the AIJ. Thus, estrogen both augments resistance to ovum transport at the AIJ and accelerates transport through the isthmus. In aggregate these experiments demonstrate the presence of two sites of delay of ovum passage. One, located at the ampullary-isthmic junction, appears to "open" between 24 and 48 hours after HCG and is estrogen sensitive. The second, located in the isthmus, opened 60 to 66 hours after HCG.

In addition to offering some insights into the mechanisms regulating tubal ovum transport, these experiments offer promise for the development of a clinically useful test of tubal function. We have demonstrated that microspheres of an appropriate size, in this case 600 μ, accurately imitate the detailed time-course of transport of natural ova. Moreover, a drug treatment known to delay the transport of natural ova also delays the transport of the microspheres, and the site of delay is identical to that shown for natural ova in our previous work.[4] Therefore, 600 μ ovum surrogates are a useful tool for studying ovum transport in the rabbit. If microspheres can be designed that accurately imitate the time-course of human ovum transport, they could be introduced into the Fallopian tubes under laparoscopic control. Stainless steel Geiger tubes that can be introduced into the uterus without dilating the cervix are commercially available. Thus, one could detect the presence of the radioactive ovum surrogate as it entered the uterine cavity, and could thereby measure the total time elapsed from placement of surrogates into the ampulla to their arrival in the uterus. Although still unproved in human subjects, this method offers the potential of measuring the physiologic competence of the oviduct to transport ovum models, and would presumeably be a valuable addition to the clinical evaluation of infertility.

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Discussion

Dr. Jack N. Blechner, Farmington, Connecticut. This paper by Drs. Pauerstein and Hodgson is both refreshing and enlightening. I wish to express my thanks for having received it in time for detailed discussions with my colleagues, Dr. Daniel Riddick and Mr. Winston Campbell.

The authors present an approach that adds a new dimension, that of transport, to the knowledge previously obtained by blowing carbon dioxide through the oviducts or by looking at contrast material in them radiographically. In this study they employed radioactive surrogate ova of various sizes and noted their transport rates in rabbit oviducts. They also studied surrogate transport of 200 μ and 600 μ models under high-dose estrogen influence. Their results showed transport of 100 μ and 200 μ surrogates at a very accelerated rate, reaching the uterus by 24 to 48 hours after an induced ovulation using HCG injections. Although the 400 μ surrogates showed slightly slower

rates, they did reach the uterus before the usual 72 hours. The 600 μ surrogates seemed to be most like normal ova in their transport rates. The 800 μ and 1,000 μ surrogates never entered the uterus during the time of study, and the 200 μ and 600 μ surrogates under estrogen influence showed an expected transport delay.

In attempting to explain these differences in transport rates, the authors related them to surrogate size. However, rabbit ova fall in the size range of 75 to 150 μ in diameter, and the previous studies of ovum transport using ovum models in the range of 120 to 160 μ in diameter[1, 3] did not show accelerated rates of transport. In one study spheres with an average size of 160 μ were obtained from the middle third of the genital tracts of mated rabbits 48 hours after placement in the tube. Why should surrogates in the same size range as normal ova show an accelerated rate of transport; yet surrogate ova three to four times as large show normal transport rates? Perhaps differences between surrogate and normal ova influence transport rates. The present study utilized plastic surrogates, whereas previous studies used Sephadex or Permutit resin particles.[3] Resin particles collect a mucin coat during their transport just as would normal ova, changing the size of the resin particle. The authors did not mention whether mucin coating occurred with plastic surrogates. If mucin deposition did not occur, the large size plastic surrogates might show normal transport, comparable to normal ova with a mucin coat that may approach 600 μ in diameter. The thickness of the mucin coat that normal ova receive during transport may be equal to or greater than the diameter of the egg itself,[5] but there is no indication that normal ova with their mucin coat do reach 600 μ in diameter. It would be interesting to know whether or not plastic surrogates collect mucin.

Surrogate ova in this study were inserted 6 hours after administration of HCG. Was their transport the same as the transport of the biologic ova released by the injection of the HCG? Did the authors observe the location of the biologic ova when the surrogate ova were located? If the data are not available, perhaps they would be worthwhile in a future experiment.

The possibility that plastic ovum models could be useful in the clinical evaluation of infertility is intriguing. If standard transport rates can be defined, significant deviations might indicate some abnormality of oviducal function. The authors provide evidence that surrogates of normal ovum size show accelerated transport, while surrogates of a larger size show normal transport rates under normal hormonal conditions. These facts may provide pitfalls in using an abnormally sized ovum model to study the physiologic functioning of the oviduct. The mucin coating of ova may act as a means of "ending the fertilizable life of the ovum."[2, 4] It has also been cited[4] that in cases where there is a reduction of the mucin coat thickness, the blastocyst does not appear viable in the uterus. The physiologic mucin coating ability of the oviduct may be an important tubal factor that cannot be studied by transport considerations alone. Nevertheless, indications of abnormal tubal transport time by the surrogate method could provide new insights into tubal transport phenomena.

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DR. PAUERSTEIN (Closing). Dr. Blechner has of course raised the critical questions. I do have some of the answers since we were concerned about the same questions.

In a paper that Dr. Hodgson and I have recently submitted for publication,[1] actual measurements were made on 15 to 35 natural ova at each of several time intervals. When one looks at the very largest ovum, which is at 60 hours, the maximum size obtained is 345 microns. It becomes smaller at 66 hours because the mucin layer diminishes. If one looks at the surrogates, it is seen that although the surrogates do acquire a mucin layer, it is never anywhere near the magnitude of that acquired by natural ova. One can see very clearly that this 596 micron surrogate which has a total size of 638 microns with mucin layer is very huge in comparison to the natural ovum.

We speculated about why this should be so, and about the corollary question of why should a model that is the same size as a natural egg go through so quickly, which is even more intriguing. We have a biophysical model, and we are adding data to it as we acquire information. Since density is a very insignificant parameter in transport, our best guess is that the compliance of the natural egg is so much greater that natural eggs get transported normally at this size but rigid surrogates do not. This also explains some of the differences in Croxatto's and Harper's studies using Sephadex and Permutit. The compliance is greater. We are looking at variables such as compliance, and surface, and density.

Your other question was whether we ever looked at the normal ova at the same time. Yes. We did not in this study because we have no way of doing it. You cannot visualize them in the intact oviduct, but we did it in a study that is in press where we killed animals at intervals and cleared the tubes and saw where the ova were and where the surrogates were, and it is fascinating because in every case the natural ova are where the surrogates are. If you use small surrogates and they get in the uterus quickly, so will the natural ova. If you use big surrogates, the natural ova are where the surrogates are. So, there is something to this gating which is all or none, and when the gate is open it is open and it lets everything through. We are working on this aspect.

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The significance of urinary free cortisol and progesterone in normal and anencephalic pregnancy

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Urinary free cortisol and progesterone were determined by radioimmunoassay in 18 normal and 16 anencephalic pregnancies and urinary free cortisol levels in 9 nonpregnant women. In normal pregnancy the urinary free cortisol (46.89 ± 34.02 μg per 24 hours) was significantly higher ($P < 0.001$) than that found with anencephaly (17.19 ± 13.20 μg per 24 hours) and 2½ times ($P < 0.001$) the nonpregnant value (18.47 ± 5.44 μg per 24 hours). In 12 of the anencephalic pregnancies, urinary free cortisol levels (11.05 ± 5.56 μg per 24 hours) were significantly lower than in nonpregnant women ($P < 0.001$). Urinary progesterone levels in normal pregnancy (15.57 ± 9.66 μg per 24 hours) and anencephaly (18.54 ± 12.69 μg per 24 hours) were comparable. The cortisol excretion values associated with anencephaly indicate that the normal fetus contributes substantially to the maternal plasma cortisol pool. Urinary free cortisol determinations may be a useful index of fetal adrenal dysfunction.

WE HAVE previously reported urinary steroid excretion patterns during pregnancy in adrenalectomized women[1] and in normal and anencephalic pregnancy before, during, and subsequent to dexamethasone administration.[2, 3] Pregnancy associated with an anencephalic fetus represents a unique biologic system for the evaluation of the maternal contribution to steroidogenesis. In anencephaly, the fetal zone of the adrenals is absent or markedly atrophic.[4, 5] Thus, the importance of the fetal adrenal gland in providing the C_{19} steroid precursors, specifically dehydroepiandrosterone sulfate (DHAS), for the formation of estrogens in the placenta is evident from the observation of low maternal urinary estriol values[3, 6] and low levels of DHAS and 16α-hydroxy-DHAS in cord blood.[7, 8]

Corticosteroid production by the fetus has recently been reported by us.[9] By multiple stepwise linear regression analyses of data obtained from plasma samples at abdominal delivery, it was demonstrated that umbilical arterial and venous cortisol and cortisone are of fetal origin. Similarly, at vaginal delivery, it was found that the umbilical venous and maternal venous cortisone levels are interdependent, whereas cortisol is primarily derived from the fetus.[9]

During the latter weeks of a normal pregnancy the maternal concentrations of total plasma cortisol are significantly elevated. It is now accepted that the determination of free unconjugated urinary cortisol is a valuable index of the concentration of free non–protein-bound plasma cortisol. The latter represents the amount of cortisol which is exposed to the tissue cells. The present communication compares normal

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and anencephalic pregnancies with respect to the urinary excretion levels of free cortisol and progesterone.

Materials and methods

This study includes nine normal nonpregnant volunteers, 18 normal pregnant volunteers, and 16 women in whom radiologic examination had confirmed the presence of an anencephalic fetus. The women in the latter group were admitted to the National Maternity Hospital in Dublin. In both pregnant groups the studies were undertaken during the last trimester of pregnancy.

Two to five consecutive 24 hour urines were collected from each pregnant woman. The normal pregnant group furnished 25 collections and 51 collections were obtained in the anencephaly group. The nonpregnant group furnished 20 collections of 24-hour urines during the follicular and luteal phases of the menstrual cycle. Urine specimens were refrigerated during collection without added preservative and stored at −15° C. until analyzed.

Radioimmunoassays (RIA). Antibodies for cortisol and progesterone were raised in rabbits with cortisol 21-hemisuccinyl-BSA and progesterone 11α-hemisuccinyl-BSA, respectively, following the procedures as described elsewhere.[10, 11]

Cortisol (RIA). Urine (0.1 ml.) was diluted to 1.0 ml. with deionized water and a tracer amount of ^{3}H-cortisol (900 c.p.m. equivalent to 2.5 pg.) was added for recovery purposes.

Extraction and thin-layer partition chromatography were carried out as previously described.[12] Radioimmunoassay was performed as described by Ruder and associates.[11]

A number of steroids were found to significantly cross-react with the antibody and, for this reason, chromatographic purification was deemed necessary.[11, 12] Interassay coefficient of variation (n = 20) was 10 per cent as obtained by the addition of authentic standard in amounts ranging between 10 and 20 ng. per milliliter of urine. Intraassay coefficient of variation (n = 30) was 8 per cent at the 20 μg per 24 hour level.

For the purpose of comparison, urinary free cortisol was measured by competitive protein binding (CPB) as described by Beardwell and associates[13] with a modification to remove progesterone by preliminary extraction with an equal volume of petroleum ether (b.p. 40 to 60°).

Progesterone (RIA). Urine (0.05 ml.) was diluted to 1.0 ml. with deionized water. A tracer amount of ^{3}H-progesterone (800 c.p.m. equivalent to 2.0 pg.) was added for recovery purposes. Then 4 ml. of petroleum ether (b.p. 40 to 60°) were added to each tube and all tubes were vortexed for 30 seconds. The aqueous layer was discarded and the petroleum ether was washed with 0.5 ml. of 8 per cent sodium bicarbonate solution followed by 0.5 ml. of deionized water. The petroleum ether was transferred to small culture tubes and evaporated to dryness under nitrogen. Then 1 ml. of acetone was added to each tube to dissolve the residue, an aliquot was taken, and its radioactivity content was determined for use in correcting the procedural losses. The radioimmunoassay was performed as described elsewhere.[14]

The characteristics of the antibody were similar to that described by Thorneycroft and Stone.[14] There was no significant cross-reaction with progesterone derivatives or any of the corticosteroids, which allowed assay of the extract without further purification. Preliminary studies with 13 different samples did not yield statistically significant results with (13.77 ± 6.25 μg per 24 hours) and without (12.15 ± 6.00 μg per 24 hours) LH-20 Sephadex column chromatography with the system described by Macdonald and associates.[15]

The interassay coefficient of variation (n = 7) was

Table I. Urinary excretion (micrograms per 24 hours) of cortisol and progesterone*

Type of subject (No. of subjects)	*Cortisol*	*Progesterone*
Normal pregnancy (18)		
Mean ± S.D.	46.89 ± 34.02	15.57 ± 9.66
Range	16.9-156.7	4.9-47.1
Anencephalic pregnancy (16)		
Mean ± S.D.	17.19 ± 13.20	18.54 ± 12.69
Range	3.3-64.6	2.6-48.1
Nonpregnancy (9)		
Mean ± S.D.	18.47 ± 5.44	
Range	5.8-29.9	
Follicular phase (7)		
Mean ± S.D.	17.19 ± 5.89	
Range	5.8-23.2	
Luteal phase (7)		
Mean ± S.D.	20.05 ± 4.70	
Range	14.5-29.9	

*Cortisol:
Anencephaly vs. normal pregnancy
$t = 5.38$; $P < 0.001$.
Anencephaly vs. non-pregnancy
$t = 0.41$; N.S.
Normal pregnancy vs. non-pregnancy
$t = 5.07$; $P < 0.001$.
Progesterone:
Anencephaly vs. normal pregnancy
$t = 1.02$; N.S.

Table II. Urinary excretion (micrograms per 24 hours) of cortisol (F) and progesterone (P) in four anencephalic pregnancies showing high cortisol excretion

	Day 1		*Day 2*		*Day 3*		*Day 4*		*Mean ± S.D. (range)*	
Subject	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
K. I.	40.67	7.70	31.28	4.47	42.58	2.32	42.28	6.50	39.20 ±5.35 (31.3-42.6)	5.25 ±2.36 (2.3-7.7)
L. A.	11.13	13.78	64.61	13.73	39.92	9.10	9.53	12.70	31.30 ±26.23 (9.5-64.6)	12.33 ±2.21 (9.1-13.8)
D. U.	28.63	8.23	4.44	12.05	35.90	30.55			22.99 ±16.47 (4.4-35.9)	16.94 ±11.94 (8.2-30.6)
M. U.	22.47	42.50	37.22	20.52	40.37	15.80			33.35 ±9.56 (22.5-40.4)	26.27 ±14.25 (15.8-42.5)

Table III. Urinary excretion (micrograms per 24 hours) of cortisol and progesterone in 12 anencephalic pregnancies*

Type of subject	*Cortisol*	*Progesterone*
Anencephalic pregnancy		
Mean ± S.D.	11.05 ± 5.56	20.07 ± 13.43
Range	3.3-23.8	2.6-48.1

*Not including four subjects listed in Table II.
Cortisol:
vs. normal pregnancy
$t = 6.18$; $P < 0.001$.
vs. nonpregnancy
$t = 4.76$; $P < 0.001$.
Progesterone:
vs. normal pregnancy
$t = 1.73$; N.S.

Table IV. Comparison of urinary cortisol values (micrograms per 24 hours) with and without thin-layer chromatography (TLC)*

Type of subject (No. of subjects)	*TLC*	*Non-TLC*
Nonpregnancy (10)		
Mean ± S.D.	11.03 ± 6.42	21.29 ± 10.76
Range	(4.6-25.0)	(9.4-43.0)
Normal pregnancy (10)		
Mean ± S.D.	26.03 ± 19.72	97.83 ± 36.47
Range	(5.6-67.5)	(40.1-153.9)

*TLC vs. non-TLC:
Nonpregnancy: $t = 2.45$; $P < 0.02$.
Normal pregnancy: $t = 5.74$; $P < 0.001$.

9.5 per cent for a level of 0.35 μg per 24 hours. The intra-assay coefficient of variation (n = 20) was 5.4 per cent for a level of 2.23 μg per 24 hours.

Results

The mean urinary excretion values for cortisol in normal and anencephalic pregnancies as well as nonpregnant females are presented in Table I. The levels in pregnancy were 2.5-fold greater than those found in the nonpregnant women. There was no difference in urinary free cortisol in nonpregnant women between the follicular and luteal phases of the menstrual cycle (Table I).

In pregnancies associated with an anencephalic fetus the mean excretion of cortisol is comparable to that found in the nonpregnant woman. However, as depicted in Table II, four individuals with anencephalic pregnancy had urinary levels of free cortisol comparable to the lower range found in normal pregnancy. If the values derived from these subjects are excluded (Table III) then there is a significant difference ($P < 0.001$) between anencephalic pregnancy and nonpregnant women. The urinary excretion of progesterone in normal and anencephalic pregnancies is also given in Table I. The progesterone values are not statistically different for the two groups even though the mean excretion value in anencephalic pregnancy is somewhat higher.

Comment

The excretion values for free cortisol (Table I) for normal pregnancy and for nonpregnant individuals are about half the levels previously reported.[16, 17] This difference may be ascribed to the fact that these investigators assayed unpurified urinary extracts. Our method includes thin-layer chromatographic purification which yields significantly lower values (Table IV). Indeed, the present TLC purification procedure

Table V. Comparison of urinary cortisol values (micrograms per 24 hours) between thin-layer chromatography (TLC) and LH-20 Sephadex column chromatography*

No. of paired assays†	*TLC*	*Column*
10		
Mean ± S.D.	66.74 ± 7.43	111.73 ± 11.18
Range	56.5-78.6	95.9-131.0

*TLC vs. column: $t = 10.06$; $P < 0.001$.
†Each in duplicate.

Table VI. Comparison of urinary cortisol values (micrograms per 24 hours) between radioimmunoassay (RIA) and competitive protein binding (CPB)*

No. of paired assays†	*RIA*	*CPB*
10		
Mean ± S.D.	28.23 ± 33.61	27.12 ± 30.31
Range	9.1-94.2	10.9-88.3

*RIA vs. CPB: $t = 0.74$; N.S.
†Each in duplicate.

furnishes values even lower (Table V) than the LH-20 Sephadex column chromatographic system described by Ruder and associates.[11] Following TLC purification, the assay carried out either by RIA or CPB gives similar results (Table VI).

In late pregnancy the concentrations of total plasma cortisol are significantly elevated in comparison with the nongestational state.[9] It is difficult, however, to draw conclusions from measurements of total plasma cortisol as to the extent of tissue exposure since only the non–protein-bound fraction is biologically active. The measurement of urinary free cortisol provides such information since it is the unbound fraction being excreted.[18] The 2½-fold increase in the urinary excretion of free cortisol in late pregnancy compared with the nongravid state in the present study are in accord with other reports.[16, 19–21] The present findings also provide evidence of increased and sustained exposure of tissues in late pregnancy to cortisol which may explain such pregnancy associated phenomena as striae gravidarum, impaired carbohydrate tolerance, hypertension, fluid retention, and improvement in any pre-existing rheumatoid arthritis.

The mean excretion of cortisol in anencephalic pregnancy is significantly lower ($P < 0.001$) than that observed in normal pregnancy. This would suggest that the fetus contributes significantly to the maternal cortisol pool. Such a contribution is sufficient to increase the non–protein-bound fraction in the plasma which is reflected by the urinary excretion. It should be noted that the urinary total 17-ketosteroids in these pregnancies (3.47 mg. per 24 hours) are significantly lower ($P < 0.01$) than those observed in normal pregnancies (7.34 ± 3.11 mg. per 24 hours), implying that a certain percentage of the maternal total urinary Zimmermann chromogens are of fetal origin.[22] Moreover, Heys and Oakey[23] have also reported significantly lower excretion of 17-ketogenic steroids in pregnancies associated with an anencephalic fetus.

Measurements of cortisol concentration in umbilical cord plasma, in a few instances of parturient women who had previously undergone total adrenal ablation, imply cortisol production by the fetal adrenal.[24] More convincing evidence was obtained from three pregnancies in a totally adrenalectomized woman where the secretion rate of cortisol by the mother prior to delivery was of the same order of magnitude as that in her infants studied within the first 3 days of life.[1]

It should be noted that the day-night variation of unbound cortisol is reduced in late pregnancy.[16, 20] According to Burke and Roulet,[16] in late pregnancy the mean plasma unbound cortisol is 1.41 μg at 9 A.M. and 0.64 μg at midnight, whereas in normal subjects the 9 A.M. value is 0.66 μg, which decreases to 0.18 μg per 100 ml. at midnight. Such an increase in the unbound fraction would be expected to lower the ACTH level in the maternal blood. Indeed, Mukherjee and Swyer[25] have recently shown that the ACTH level in pregnant women is significantly lower than that found in nonpregnant women. The fetal involvement in the maternal excretion of cortisol is also signified by the fact that in severe rhesus isoimmunizations, urinary estriol and free cortisol bear a direct relationship.[17] It is well established that urinary estriol is intimately involved with the functional status of the fetal adrenals. It should be emphasized, however, that the fetal contribution of cortisol is not sufficient to sustain maternal needs, as supplementation therapy is required in pregnant patients who have undergone total adrenal ablation or have adrenal insufficiency.

The free urinary cortisol levels in four pregnancies associated with anencephalic infants were within the low-normal range (Table II). Shahwan and associates[26] demonstrated the ability of adrenal tissue from anencephalic infants to convert pregnenolone and progesterone to cortisol and corticosterone in vitro. These authors noted that the adrenal glands of the anencephalics in their study were composed almost entirely of definitive gland tissue. Consequently, it may be surmised that in these four patients the fetal definitive zone of the adrenals was relatively active. It is pertinent to note that the progesterone excretion

values in three of these subjects showed a decrease when compared with the mean values of the remaining 12 such pregnancies (Tables II and III). The significantly decreased cortisol excretion in comparison to nonpregnancy (Table III) may be due to utilization of maternal cortisol by the fetus and/or an increased level of corticosteroid-binding globulin (CBG). Although estrogen production is lower in anencephalic pregnancy than normal pregnancy, it is still higher when compared with values encountered during the normal menstrual cycle. Consequently, an increased level of CBG in anencephalic pregnancy would be expected to result in greater cortisol binding and diminished urinary excretion.

The excretion of progesterone in normal and anencephalic pregnancies is similar (Table I). This would suggest that as opposed to estrogen biosynthesis, the fetal adrenals are not involved in the biogenesis of progesterone. Our previous report[3] on the urinary excretion of pregnanediol did not reveal any difference between these two groups of pregnant women. It has been recently reported that maternal blood cholesterol is the predominant precursor of placental progesterone.[27] The comparable excretion values for progesterone in normal and anencephalic pregnancy further strengthen such a conclusion.

In view of our present data, it may be suggested that the measurement of urinary free cortisol may be useful for the diagnosis of fetal adrenal hypoplasia or hyperplasia and to monitor fetal well-being in cases of placental sulfatase deficiency where urinary estriol excretion is decreased.[28]

We thank Mr. Richard A. Bartlett for his excellent technical assistance.

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Discussion

DR. WILLIAM N. SPELLACY, Gainesville, Florida. Dr. Charles and his associates have utilized an experiment of nature to further our understanding of the glucocorticoid steroid patterns in pregnancy. Their steroid studies involved the radioimmunoassay measurements of progesterone and free cortisol in 24 hour urine specimens from nonpregnant and pregnant women, the latter with normal and anencephalic fetuses. Their free cortisol values are somewhat lower than those reported by others and this probably represents an improvement in methodology by extraction, thin-layer chromatography, and antibody binding, for in general a lowering of values accompanies a more specific assay system which no longer detects extraneous substances. Their results show that the urinary progesterone values are similar in pregnancies with normal and anencephalic fetuses. They also demonstrate a 2.5-fold increase in the normal term pregnancy daily urinary excretion of free cortisol over nonpregnant values. This was not found, however, when the fetus was anencephalic. Since daily urine free cortisol levels presumably represent an approximation of the integrated non–protein-bound free plasma cortisol concentration, these data support other blood studies showing elevated free cortisol levels in pregnant women and suggest that at least some of the cortisol excess is derived from the fetal adrenal. The results extend the umbilical cord blood studies which showed higher free cortisol levels in umbilical artery blood compared to vein blood and suggested a fetal cortisol contribution to the mother.[1] These data further our understanding of pregnancy and help to explain why conditions such as asthma, lupus erythematosus, and rheumatoid arthritis often improve during pregnancy and exacerbate in the early postpartum period.

I would like to raise five points for further discussion by Dr. Charles.

1. Since the cortisol results depend upon accurate 24 hour urine collections, how were the collections monitored? Were there measurements made of urine creatinine to document the accuracy of the collection?

2. Since Beisel and others[2] have shown that urine free cortisol is dependent upon glomerular filtration rate (GFR) and perhaps also on the renal tubular reabsorption maximum, do the authors have any data concerning the GFR in the cases studied, and do they have any information on the Tm for cortisol in pregnancy? Does some of their suggested cortisol excess reflect renal alterations as well as adrenal alterations?

3. Four of the women with anencephalic fetuses had near normal urine free cortisol excretion rates and these were excluded in Table III to then give significantly different results when compared to normal nonpregnant women. How do the authors justify these exclusions, since such a selection process would be expected to improve statistical significance?

4. The variations in central nervous system defects is recognized with the anencephalic syndrome. Do the authors have any morphologic information from neonate autopsy studies or do they have other hormone measurements such as neonatal blood TSH, hPr, ACTH or growth hormone, to suggest that the variation in the anencephalic cortisol results might be due to variation in the extent of the hypothalamic-pituitary-adrenal damage?

5. The authors conclude by suggesting that perhaps the determination of maternal urinary free cortisol levels may be helpful in assessing the status of the fetal-placental unit. A review of the results on their patients shows great variation. For example, patient L. A. shown in Table II had a marked variation in the free cortisol excretion patterns from 9 to 64 μg per day over the 4 days of study where the Infant was presumably alive and well. How much variation do the authors then expect in normal pregnancy and will this be of such a large degree as to make the interpretation of pathologic changes difficult?

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DR. CHARLES (Closing). We did measure creatinine. The most important point in this regard, however, is the expected similar excretion values obtained for progesterone in normal and anencephalic pregnancy. It has been shown by other investigators that the fetal adrenals are not involved in the biogenesis of placental progesterone. The maternal blood cholesterol is the

predominant precursor. Furthermore, these patients were put in the metabolic ward for the urinary collections.

Next, we have no data on the renal clearance rate of the patients either in the anencephalic or the normal group of pregnancies. I would like to mention, however, that there was no superimposed toxemia, hypertension, or renal abnormality in either group of patients.

The next thing is that we have some data which has not been presented in the paper as regards the anencephalic group. Among the four out of our group which showed relatively high excretion of cortisol (thanks to the Pathology Department at National Maternity, Dublin, Ireland), we found that three of these anencephalics had relatively normal adrenal glands. In other words, the fetal zone was absent, but there was a good definitive zone. This is why we took these four separately, but if these four are not taken separately as shown in our composite table, the values for urinary cortisol in the anencephalic group are similar to those obtained in the nonpregnant status.

We have some further information also as regards CNS deformities. We published some time ago EM studies on the anterior pituitary of anencephalics, and we did find in the majority of anencephalics it was not the anterior pituitary which was defective; the defect was in the hypothalamus.

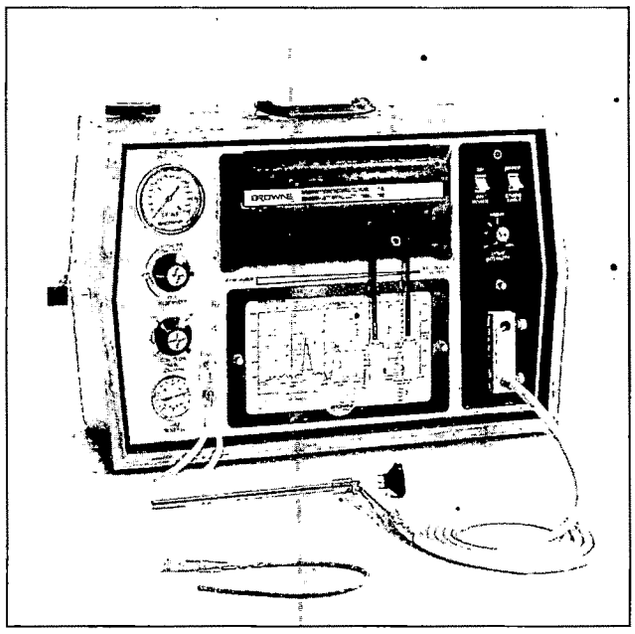
Finally, there is a great deal of day-to-day variation, and we are trying to narrow or home in on a group of patients where we feel, because of interest in respiratory distress syndrome, the cortisol measurements in the urine may be of greater value if we take appropriate measures to study a larger series of patients.

Lower Urinary Tract Dysfunction... ...Should You Operate?

One of the most critical decisions in any diagnosis today is whether or not to operate. Based on the most comprehensive diagnostic data available, you must make that recommendation. Browne Corporation CO_2 Endoscopy/Cystometry Monitors are designed to add significantly to that data and to present it to you in its most usable form. Consider these features:

Fail Safe Gas Supply System: Delivers CO_2 to patient at selected pre-calibrated flow rates. Three independent safety systems limit pressure and volume supplied during a single cycle. Medical grade cartridge powers both the supply and recording systems.

Automatic Catheter Puller: Synchronized with the recording system to provide direct readout of urethral length and position on the patient chart card.



Fiber Optic Illuminator: Dual 150 watt lamps with variable intensity and selector controls are provided as an option for endoscopy procedures.

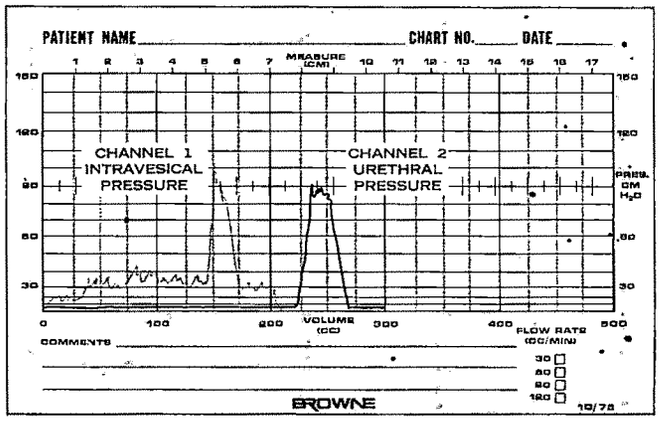
Robertson Urethrascope: Straight ahead, wide-angle field of view. Fully concentric fiber optics assure even illumination. Integral handle with fiber optic and gas fittings simplifies assembly and sterilization. Removable gas sheath, obturator and case included.

Four Function Profile Chart Recorder: Monitors total CMG volume, urethral position, intravesical pressure, and/or urethral pressure. Proprietary design is non-electrical and requires no calibration.

Permanent Record: The exclusive patient chart card provides a clear, concise, retainable record of each procedure for historical and legal purposes. Diagnosis of complex, interacting variables is often greatly simplified.

The sample card shown at right (one half actual size) is a simulation of a pressure/volume cystometrogram with simultaneous urethral pressure monitoring. Channel one indicates intravesical pressure spasms in excess of 100cm H_2O. Simultaneously, the Monitor's second channel has recorded a normal urethral pressure of 90cm H_2O. This patient's urinary incontinence is probably due to an uninhibited neurogenic bladder. A simple in-office procedure has ruled strongly against surgical candidacy.

Cystometry And Urethral Pressure Catheters: Available in both single lumen and dual lumen versions with calibrated cm markings. Designed to attach directly to the automatic catheter puller.



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Articles to appear in early issues

Scalene node biopsy in the pretreatment staging of carcinoma of the cervix uteri
Carlos Perez-Mesa, M.D., and John S. Spratt, Jr., M.S.P.H., M.D., F.A.C.S.
Columbia, Missouri

Microinvasive carcinoma of the cervix
Robert M. Ruch, M.D., James A. Pitcock, M.D., and Walter A. Ruch, Jr., M.D.
Memphis, Tennessee

Immunobiological function of the syncytiotrophoblast: A new theory
Masaru Morisada, M.D., Hisao Yamaguchi, M.D., and Rihachi Iizuka, M.D.
Tokyo, Japan

Relationship between cortisol levels in umbilical cord plasma and development of the respiratory distress syndrome in premature newborn infants
S. Sybulski, M.Sc., Ph.D., and G. B. Maughan, M.Sc., M.D.
Montreal, Quebec, Canada

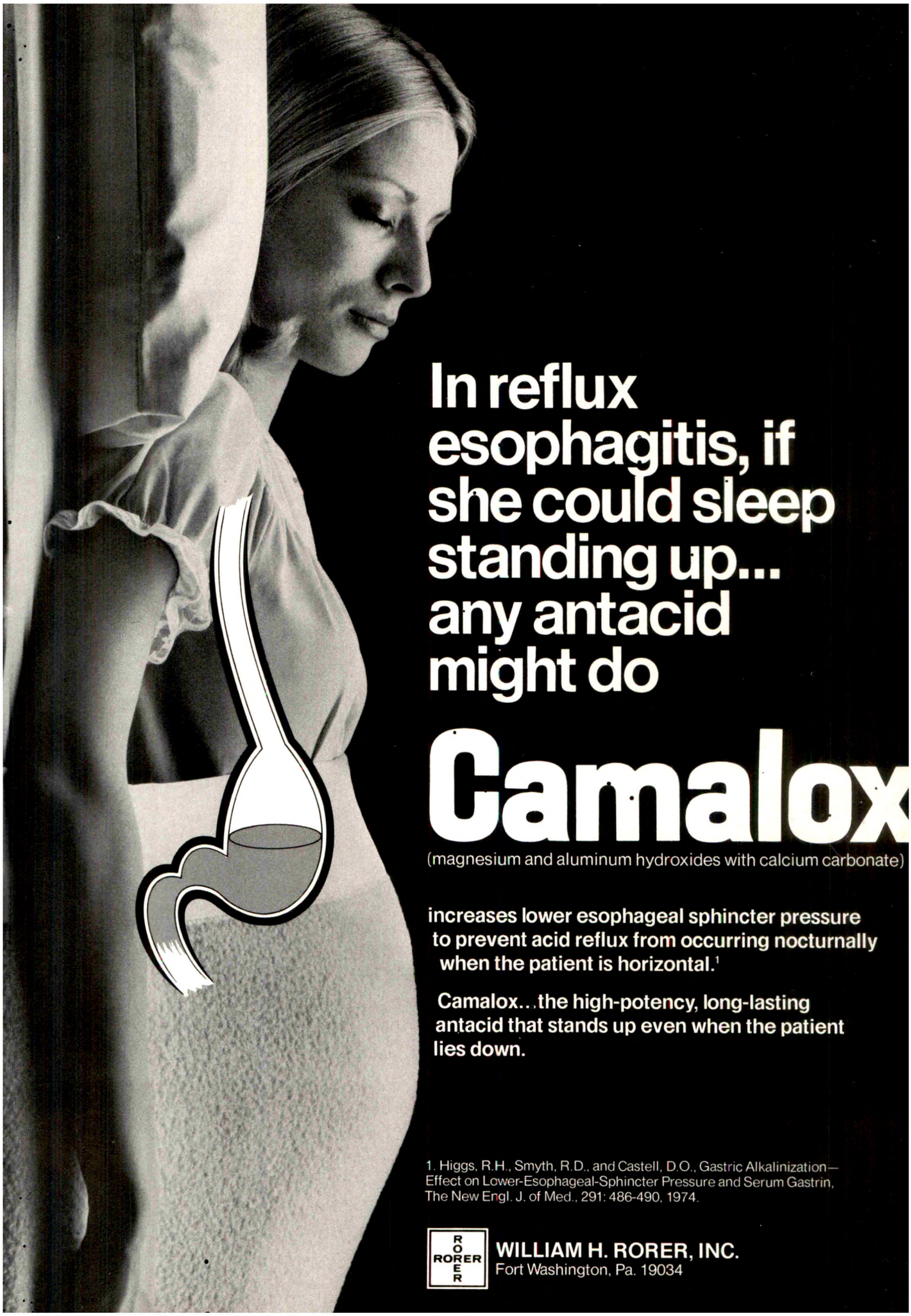
Breathing patterns before death in fetal lambs
J. E. Patrick, M.D., F.R.C.S.(C.), K. J. Dalton, B.Sc., M.B., B.S., and G. S. Dawes, F.R.S., D.M.
Headington, Oxford, England

Absolute neonatal insulin levels in children of insulin-requiring diabetic patients
F. I. R. Martin
Melbourne, Victoria, Australia

Fetal acid-base balance. I. Interdependence of maternal and fetal Pco_2 and bicarbonate concentration
Allan Chang and Carl Wood
Melbourne, Victoria, Australia

An experimental model for disseminated herpesvirus infection of the neonate
Marvin S. Amstey, M.D., and Katherine Kobos, B.A.
Rochester, New York

Incidence of infections associated with the intrauterine contraceptive device in an isolated community
Philip B. Mead, M.D., F.A.C.O.G., Jackson B. Beecham, M.D., and John Van S. Maeck, M.D., F.A.C.O.G.
Burlington, Vermont



In reflux esophagitis, if she could sleep standing up... any antacid might do

Camalox

(magnesium and aluminum hydroxides with calcium carbonate)

increases lower esophageal sphincter pressure to prevent acid reflux from occurring nocturnally when the patient is horizontal.[1]

Camalox...the high-potency, long-lasting antacid that stands up even when the patient lies down.

1. Higgs, R.H., Smyth, R.D., and Castell, D.O., Gastric Alkalinization—Effect on Lower-Esophageal-Sphincter Pressure and Serum Gastrin, The New Engl. J. of Med., 291: 486-490, 1974.

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WILLIAM H. RORER, INC.
Fort Washington, Pa. 19034

The Kantrex® (kanamycin sulfate) record:

Percent in vitro susceptible to kanamycin.*

	1970	1971	1972	1973	1974
E. coli	90.8	90.5	89.9	90.0	92.0
proteus sp.	89.5	89.5	88.4	90.0	91.0
klebsiella sp.	86.9	86.6	87.9	90.0	93.0
enterobacter sp.	90.5	90.1	90.2	91.4	92.0
serratia sp.	39.2	60.1	72.3	75.6	80.0
herellea sp.	83.2	86.8	87.0	88.4	91.0
mima sp.	78.1	73.9	76.5	74.4	78.0

*Susceptibility data from records based on over 250,000 isolates annually from 180 different hospitals. No in vivo correlation is claimed from the in vitro data.

In a world of changing bacterial challenges... a continuing record of bacterial susceptibility.

BRIEF SUMMARY OF PRESCRIBING INFORMATION

Combined Kantrex Injection and Pediatric Injection
(kanamycin sulfate injection)

For complete information, consult Official Package Circular.
(11) 6/18/74

Warning: Irreversible deafness can occur. Tinnitus or vertigo may also occur and indicate vestibular damage and impending deafness. The risk is sharply increased with renal dysfunction. In such cases, decrease size and frequency of doses. Discontinue kanamycin and check hearing if azotemia increases. Watch carefully for ototoxicity in older patients and patients receiving more than 15 Gm. of kanamycin. To avoid neuromuscular paralysis with respiratory depression, postpone intraperitoneal instillation in post-operative patients until recovery from anesthesia and muscle relaxants is complete. Although there have been isolated reports of respiratory depression following intraperitoneal instillation of kanamycin, there is no conclusive proof that this side effect can be produced with recommended doses of the drug. Avoid concurrent use of other ototoxic drugs including ethacrynic acid. Safety in pregnancy is not established.

Indications: Serious infections due to susceptible strains of E. coli, Proteus sp., E. aerogenes, K. pneumoniae, Serratia marcescens and Mima-Herellea. Culture and sensitivity studies should be performed.

Contraindications: A history of hypersensitivity to the drug. Prior auditory damage by kanamycin or other agents may be a contraindication if effective, alternative therapy is available.

Precautions: Obtain audiograms before and during therapy in patients with renal dysfunction when treatment lasts more than 5 days. Stop Kantrex if tinnitus or hearing loss occurs. Hydrate patients to prevent chemical irritation of the renal tubules. Assess renal function periodically, both before and during therapy. If signs of renal irritation occur (casts, cells, proteinuria), increase hydration and reduce the dosage or the frequency of dosage if necessary. In azotemic patients, the frequency (in hours) of doses may be obtained by multiplying the serum creatinine by 9. If azotemia or oliguria occur, discontinue therapy. Mycotic or bacterial superinfection may occur.

Adverse Reactions: Nephrotoxicity—albuminuria, presence of white and red cells, granular casts, azotemia and oliguria have been reported.

Ototoxicity—See Box Warning.

Irritation or pain at the injection site, skin rash, drug fever, headache and paresthesias.

Dosage and Administration: The usual dose is 7.5 mg./Kg./12 hours I.M. or I.V. The average adult dose is 1 Gm. daily. The maximum adult total daily dose should not exceed 1.5 Gm. by all routes of administration and the total amount administered should not exceed 15 Gms.

The intravenous route is used only if intramuscular administration is not feasible. The 0.5 Gm. dose is added to 200 ml. of sterile diluent and administered 60-80 drips/minute.

When used as intraperitoneal instillation, the above dose is diluted in 20 ml. of sterile diluent. If possible, instillation should be postponed until after patient is fully recovered from effects of anesthesia and muscle-relaxing drugs.

As aerosol treatment, 250 mg. of Kantrex is diluted with 3 ml. of physiological saline and nebulize.

Kantrex Injection in concentrations of 0.25 percent (2.5 mg./ml.) has been used satisfactorily as an irrigating solution in abscess cavities, pleural spaces, peritoneal and ventricular cavities.

Supplied: Rubber capped vials as a ready-to-use sterile aqueous solution in two concentrations: 0.5 Gm. in 2 ml., 1.0 Gm. in 3 ml.

Pediatric Injection 75 mg. in 2 ml.

BRISTOL

BRISTOL LABORATORIES
Division of Bristol-Myers Co.
Syracuse, New York 13201

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Most women who start with it stay with it



Ortho-Novum 1/50 TRADEMARK □21 and 28

Each yellow tablet contains 1 mg norethindrone and 0.05 mg mestranol. Each green tablet in the 28-day regimen contains inert ingredients.

Effective low hormonal dosage

Each tablet contains a low dosage of progestin, 1 mg norethindrone, and a low dosage of estrogen, 0.05 mg mestranol. Clinical studies with Ortho-Novum 1/50 show a pregnancy rate of only 0.26 per 100 woman years.

Low incidence of minor side effects

Experience shows a low incidence of discontinuance due to medication. Serious as well as minor side effects have been reported following the use of oral contraceptives. These include thromboebolic disease. The physician should remain alert to the earliest manifestations of any symptoms of serious disease and discontinue oral contraceptive therapy when appropriate. The physician should be fully aware of the complete Prescribing Information for this product.

Dialpak* Tablet Dispenser

Designed for your patients' convenience, but more importantly, helps reduce the possibility of error through missed tablets. The unique DIALPAK dispensing system is patient preferred.†

*TRADEMARK †Burke Marketing Research, Inc.

Most women who start with it stay with it



Ortho-Novum 1/50 □21 and 28

TRADEMARK

Ortho-Novum 1/50 □21 and Ortho-Novum 1/50 □28

Description: Each ORTHO-NOVUM 1/50 □21 Tablet contains 1 mg of the progestational substance, norethindrone, together with 0.05 mg of the estrogenic compound, mestranol.

Each yellow ORTHO-NOVUM 1/50 □28 Tablet contains 1 mg of the progestational substance, norethindrone, together with 0.05 mg of the estrogenic compound, mestranol. Each green tablet contains inert ingredients.

Actions: ORTHO-NOVUM 1/50 □21 and ORTHO-NOVUM 1/50 □28 act through the mechanism of gonadotrophin suppression, i.e., through the estrogenic and progestational actions of the ingredients. Alterations in cervical mucus and in the endometrium may also contribute to the efficacy of ORTHO-NOVUM 1/50 □21 and ORTHO-NOVUM 1/50 □28.

Special note: Oral contraceptives have been marketed in the United States since 1960. Reported pregnancy rates vary from product to product. The effectiveness of the sequential products appears to be somewhat lower than that of the combination products. Both types provide almost completely effective contraception.

The physician should consider a thorough discussion with each patient about the prescribing information, risks and benefits associated with oral contraceptives, taking into account various factors such as age and other physical conditions.

An increased risk of thromboembolic disease associated with the use of hormonal contraceptives has now been shown in studies conducted in both Great Britain and the United States. Other risks, such as those of elevated blood pressure and reduced tolerance to carbohydrates, have been reported and appropriate tests should be conducted to monitor these during oral contraceptive therapy. Liver disease has also been reported, and the physician should be alert to its earliest manifestations.

Long-term administration of both natural and synthetic estrogens in subprimate animal species in multiples of the human dose increases the frequency for some animal carcinomas. These data cannot be transposed directly to man. The possible carcinogenicity due to the estrogens can neither be affirmed nor refuted at this time. Close clinical surveillance of all women taking oral contraceptives must be continued.

Indication: Contraception.

Contraindications: 1. Thrombophlebitis, thromboembolic disorders, cerebral vascular disease, myocardial infarction, or a past history of these conditions. 2. Markedly impaired liver function. 3. Known or suspected carcinoma of the breast. 4. Known or suspected estrogen-dependent neoplasia. 5. Undiagnosed abnormal genital bleeding. 6. Known or suspected pregnancy.

Warnings: 1. The physician should be alert to the earliest manifestations of thrombotic and thromboembolic disorders, thrombophlebitis, cerebrovascular disorders including hemorrhage, pulmonary embolism and retinal thrombosis. Should any of these occur or be suspected, the drug should be discontinued immediately.

Retrospective studies of morbidity and mortality in Great Britain and studies of morbidity in the United States have shown a statistically significant association between thrombophlebitis, pulmonary embolism and cerebral vascular disease, occlusive or hemorrhagic, and the use of oral contraceptives. There have been three principal studies in Great Britain[1-3] leading to these conclusions and three in this country.[4-7] The estimate of the relative risk of thromboembolism in the study by Vessey and Doll[3] was about sevenfold, while the United States studies found relative risks of 4.4 to 11, meaning that the users are several times as likely to undergo thromboembolic disease without evident cause as nonusers.

In May, 1974, the Royal College of General Practitioners in England[8] issued an interim report of its continuing large-scale prospective study comparing a user group to a nonuser group. This study in its interim analysis states, "A statistically significant higher rate of reporting of cerebrovascular accidents in Takers is evident, but the numbers are too small to justify an estimation of the degree of risk." The study also reported a higher incidence of superficial and deep vein thrombosis in users as compared to nonusers. The risk of superficial and deep vein thrombosis was reported to be lower in women using 50 mcg estrogen preparations.

The Sartwell study[4] indicated that the risk did not persist after discontinuation of administration. Both the Sartwell and the Royal College studies indicated that the degree of risk was not associated with duration of treatment.

In a collaborative American study[5,6] of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the relative risk of thrombotic stroke was 4.1 to 9.5 times greater in users than in nonusers. A comparable estimate for hemorrhagic stroke was 2.0.

None of the American studies was designed to evaluate a difference between products. However, the Sartwell study[4] suggested that there might be an increased risk of thromboembolic disease in users of sequential products.

Other retrospective studies[9,10] have reported an increased risk of post-surgery thromboembolic complications in oral contraceptive users. It has been recommended that therapy be discontinued at least one month prior to elective surgery.

In May of 1975 two separate retrospective case control studies relating to fatal and nonfatal myocardial infarction in users and nonusers of oral contraceptives were reported.[11,12] The studies estimate that the increased risk of hospital admission for myocardial infarction associated with the use of oral contraceptives as compared to nonusers is 2.7 times in the 30-39 age group and 5.7 times in the 40-44 age group; and the risk of death associated with the use of oral contraceptives as compared to nonusers was increased 2.8 times for users in the 30-39 age group and 4.7 times in the 40-44 age group. One of these studies reported that oral contraceptive users who had myocardial infarction were more likely to be heavy smokers and have certain additional risk factors, i.e., treated hypertension and diabetes, pre-eclamptic toxemia, obesity and type II hyperlipoproteinemia.

2. Discontinue oral contraceptive medication if there is: gradual or sudden partial or complete loss of vision; proptosis or diplopia; onset or aggravation of migraine or development of headache of a new pattern which is recurrent, persistent or severe; papilledema; or any evidence of retinal vascular lesions.

3. Fetal abnormalities have been reported to occur in the offspring of women who have taken progestogens and/or estrogens during pregnancy.[13,14] The safety of ORTHO-NOVUM 1/50 □21 or ORTHO-NOVUM 1/50 □28 in pregnancy has not been demonstrated. Pregnancy should be ruled out before initiating or continuing the contraceptive regimen. Pregnancy should always be considered if withdrawal bleeding does not occur.

4. A small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The long-range effect to the nursing infant cannot be determined at this time.

5. Hepatic lesions (adenomas, hepatomas, hamartomas, regenerating nodules, etc.), occasionally fatal, have been reported in women on oral contraceptives. Such lesions may present as an abdominal mass or with the signs and symptoms of an acute abdomen. These lesions should be considered if the patient has abdominal pain or evidence of intra-abdominal bleeding. This has been reported in short-term as well as long-term users of oral contraceptives.

Precautions: 1. A thorough history and physical examination should be performed before prescribing oral contraceptives and periodically during their administration and should include special reference to breasts and pelvic organs, including Papanicolaou smear since estrogens have been known to produce tumors, some of them malignant, in five species of subprimate animals. 2. Endocrine and possibly liver function tests may be affected by treatment with ORTHO-NOVUM 1/50 □21 or ORTHO-NOVUM 1/50 □28. Therefore, if such tests are abnormal in a patient taking ORTHO-NOVUM 1/50 □21 or ORTHO-NOVUM 1/50 □28, it is recommended that they be repeated after the drug has been withdrawn for two months. 3. Under the influence of estrogen-progestogen preparations, pre-existing uterine fibromyomata may increase in size. 4. Because these agents may cause some degree of fluid retention, conditions which might be influenced by this factor, such as epilepsy, migraine, asthma, cardiac or renal dysfunction, require careful observation. 5. In breakthrough bleeding, and in all cases of irregular bleeding per vaginam, nonfunctional causes should be borne in mind. In undiagnosed bleeding per vaginam, adequate diagnostic measures are indicated. 6. Patients with a history of psychic depression should be carefully observed and the drug discontinued if the depression recurs to a serious degree. 7. Any possible influence of prolonged ORTHO-NOVUM 1/50 □21 or ORTHO-NOVUM 1/50 □28 therapy on pituitary, ovarian, adrenal, hepatic or uterine function awaits further study. 8. A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. The mechanism of this decrease is obscure. For this reason, diabetic patients should be carefully observed while receiving ORTHO-NOVUM 1/50 □21 or ORTHO-NOVUM 1/50 □28 therapy. 9. The age of the patient constitutes no absolute limiting factor, although treatment with ORTHO-NOVUM 1/50 □21 or ORTHO-NOVUM 1/50 □28 may mask the onset of the climacteric. 10. The pathologist should be advised of ORTHO-NOVUM 1/50 □21 or ORTHO-NOVUM 1/50 □28 therapy when relevant specimens are submitted. 11. Susceptible women may experience an increase in blood pressure following administration of contraceptive steroids. 12. Cholestatic jaundice has been reported in users of oral contraceptives. If this occurs, ORTHO-NOVUM 1/50 □21 or ORTHO-NOVUM 1/50 □28 should be discontinued. This condition is more likely to occur in patients who have experienced cholestatic jaundice of pregnancy. Patients with a history of cholestatic jaundice of pregnancy should be carefully observed during ORTHO-NOVUM 1/50 □21 or ORTHO-NOVUM 1/50 □28 therapy.

Adverse reactions observed in patients receiving oral contraceptives: A statistically significant association has been demonstrated between use of oral contraceptives and the following serious adverse reactions: thrombophlebitis, pulmonary embolism, cerebral thrombosis and hemorrhage, myocardial infarction, gallbladder disease.

Although available evidence is suggestive of an association, such a relationship has been neither confirmed nor refuted for the following serious adverse reactions: neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis; hepatic lesions with or without intra-abdominal bleeding.

The following adverse reactions are known to occur in patients receiving oral contraceptives: nausea, vomiting, gastrointestinal symptoms (such as abdominal cramps and bloating), breakthrough bleeding, spotting, change in menstrual flow, amenorrhea during and after treatment, edema, chloasma or melasma, which may persist, cholestatic jaundice, migraine, rash (allergic), mental depression, change in weight (increase or decrease), breast changes (tenderness, enlargement and secretion), changes in cervical erosion and cervical secretions, suppression of lactation when given immediately post-partum, rise in blood pressure in susceptible individuals.

Although the following adverse reactions have been reported in users of oral contraceptives, an association has been neither confirmed nor refuted: anovulation post-treatment, which tends to occur more frequently in women with a history of menstrual disorders, premenstrual-like syndrome, changes in libido, changes in appetite, cystitis-like syndrome, headache, intolerance to contact lenses, nervousness, dizziness, fatigue, backache, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruptions, itching, vaginitis.

The following laboratory results may be altered by the use of oral contraceptives: hepatic function—increased sulfobromophthalein retention and other tests; coagulation tests—increase in prothrombin, Factors VII, VIII, IX and X; decrease in anti-thrombin III; increase in platelet aggregability; thyroid function—increase in PBI, and butanol-extractable protein-bound iodine and decrease in T^3 uptake values; metyrapone test; pregnanediol determination.

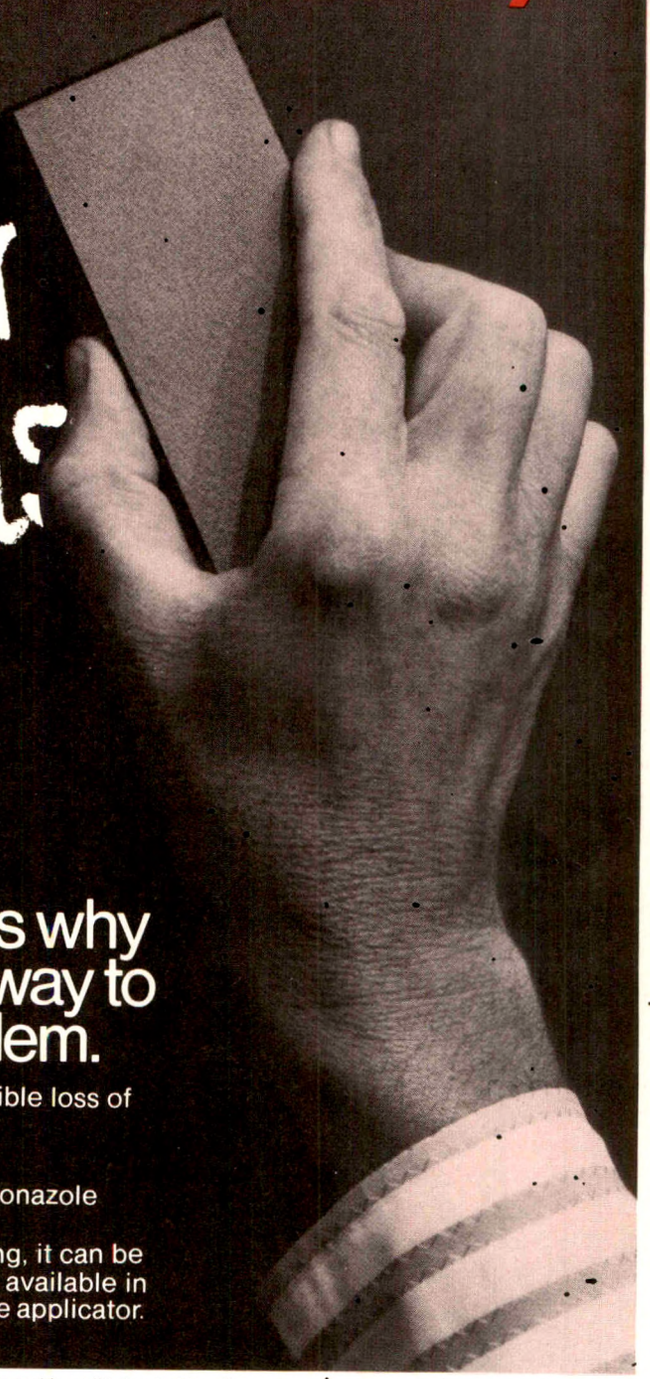
References: 1. Royal College of General Practitioners: Oral Contraception and Thrombo-Embolic Disease. J Coll Gen Pract *13*:267-279, May 1967. **2.** Inman, W.H.W.; Vessey, M.P.: Investigation of Deaths from Pulmonary, Coronary and Cerebral Thrombosis and Embolism in Women of Child-Bearing Age, Br Med J *2*:193-199, April 27, 1968. **3.** Vessey, M.P.; Doll, R.: Investigation of Relation between Use of Oral Contraceptives and Thromboembolic Disease. A Further Report, Br Med J *2*:651-657, June 14, 1969. **4.** Sartwell, P.E.; Masi, A.T.; Arthes, F.G.; Greene, G.R.; Smith, H.E.: Thromboembolism and Oral Contraceptives: An Epidemiologic Case-Control Study, Am J Epidemiol *90*:365-380, Nov. 1969. **5.** Oral Contraception and Increased Risk of Cerebral Ischemia or Thrombosis, N Engl J Med *288*(17):871-878, April 26, 1973. **6.** Oral Contraceptives and Stroke in Young Women. Associated Risk Factors, J.A.M.A. *231*(7):718-722, Feb. 17, 1975. **7.** Oral Contraceptives and Venous Thromboembolic Disease, Surgically Confirmed Gall-Bladder Disease, and Breast Tumours. Report from the Boston Collaborative Drug Surveillance Programme, Lancet: 1399-1404, June 23, 1973. **8.** Royal College of General Practitioners: Oral Contraceptives and Health, 1-100, May 1974. **9.** Vessey, M.P.; Doll, R.; Fairbairn, A.S.; Glober, G.: Postoperative Thromboembolism and the Use of Oral Contraceptives, Br Med J *3*:123-126, July 18, 1970. **10.** Greene, G.R.; Sartwell, P.E.: Oral Contraceptive Use in Patients with Thromboembolism Following Surgery, Trauma, or Infection, Am J Public Health *62*(5):680-685, May 1972. **11.** Mann, J.I.; Vessey, M.P.; Thorogood, M.; Doll, R.; Myocardial Infarction in Young Women with Special Reference to Oral Contraceptive Practice, Br Med J *2*:241-245, May 3, 1975. **12.** Mann, J.I.; Inman, W.H.W.: Oral Contraceptives and Death from Myocardial Infarction, Br Med J *2*:245-248, May 3, 1975. **13.** Nora, J.J.; Nora, A.H.: Birth Defects and Oral Contraceptives, Lancet: 941-942, April 28, 1973. **14.** Janerich, D.T.; Piper, J.M.; Glebatis, D.M.: Oral Contraceptives and Congenital Limb-Reduction Defects, N Engl J Med *291*(14):697-700, Oct. 3, 1974.

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Ortho

OJ 125-6

KOROSTATIN
...(Nystatin Tablets U.S.P.)



Vulvovagir
Candidia

There are a lot of reasons why Korostatin is the better way to eliminate a serious problem.

- NO REFRIGERATION NEEDED...Eliminates possible loss of potency at room temperature
- EFFICACY...A proven specific
- ECONOMY...Dramatically less expensive than miconazole nitrate cream
- ADDITIONAL BENEFITS...Korostatin is nonstaining, it can be used throughout pregnancy, it is well tolerated and available in convenient 14 and 28 tablet packages, with reusable applicator.

DESCRIPTION: KOROSTATIN (Nystatin) Vaginal Tablets, U.S.P. is in an oblong shaped vaginal tablet, each containing 100,000 units of Nystatin, U.S.P. Inactive ingredients include: starch, lactose, polyvinylpyrrolidone, sorbitol and magnesium stearate.

INDICATIONS FOR USE: KOROSTATIN (Nystatin) Vaginal Tablets U.S.P. are indicated in the local treatment of vulvovaginal candidiasis.

CONTRAINDICATIONS: All Nystatin preparations are contraindicated in patients with a history of hyper-sensitivity to Nystatin.

ADVERSE REACTIONS: Nystatin, U.S.P. is virtually nontoxic and nonsensitizing and is well tolerated by all age groups, even on prolonged administration. If irritation during intravaginal use should occur, discontinue the medication.

For full prescribing information, consult package insert.

KOROSTATIN is manufactured under Holland-Rantos specifications by the American Cyanamid Company.

HOLLAND-RANTOS CO., INC.
P.O. Box 5, Piscataway, New Jersey 08854
Serving the need of the OB/GYN community for over 50 years.

In serious urinary tract infections*

*due to indicated susceptible organisms



B_ for_ th_ I b t_ _ _ _ com_ back
start with 'ANCEF'

Serious urinary tract infections usually require immediate treatment. In these cases prudent medical judgment and the use of 'Ancef', when appropriate, can save valuable time while waiting for lab results. 'Ancef' offers:

- **Broad coverage of major urinary tract pathogens**
  E. coli, Proteus mirabilis, Klebsiella species and some strains of enterobacter and enterococci.
- **High urinary levels**
  'Ancef' has been found to achieve peak urine concentrations of approximately 2400 mcg/ml and 4000 mcg/ml respectively following 500 mg and 1 gram IM doses in normal adult volunteers.
- **Convenient IV or IM administration at same dosage schedule**

• When lab tests return, if the indicated organism is sensitive to 'Ancef'—and chances are it will be—therapy is already under way. If it isn't sensitive, then 'Ancef' should be discontinued and an appropriate antibiotic selected.

Adverse Reactions
Reported adverse reactions include pain on injection, rash, G.I. symptoms, pruritus and phlebitis. For a complete list of reported adverse reactions, please see the next page.

Warning: In penicillin-sensitive patients, cephalosporins should be used with caution because of evidence of partial cross-allergenicity of the penicillins and cephalosporins.

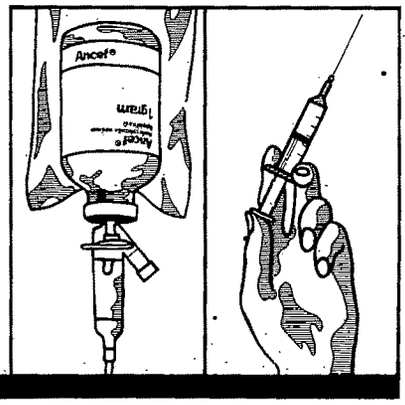
Note: In patients with impaired renal function, lower daily dosage is required.

Please see next page for a brief summary of prescribing information.

Smith Kline & French Laboratories
Division of SmithKline Corporation, Philadelphia, Pa.

ANCEF® IV IM
brand of sterile
CEFAZOLIN SODIUM
(LYOPHILIZED)

Injection: 250 mg, 500 mg and 1 gram in 10 ml vials;
1 gram, 5 grams and 10 grams in 100 ml vials.

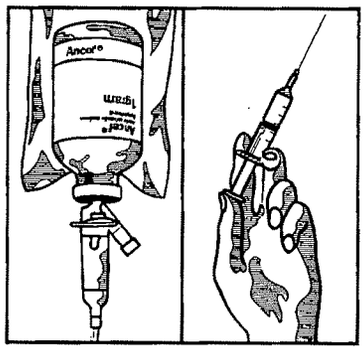


start with ANCEF® IV IM

brand of sterile

CEFAZOLIN SODIUM

(LYOPHILIZED)



Before prescribing, see complete prescribing information in SK&F literature or *PDR*. The following is a brief summary.

DESCRIPTION

Ancef® (sterile cefazolin sodium, SK&F) is a semi-synthetic cephalosporin for parenteral administration. It is the sodium salt of 3-{[(5-methyl-1, 3, 4-thiadiazol-2-yl) thio] methyl}-8-oxo-7-[2-(1H-tetrazol-1-yl) acetamido]-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid. The sodium content is 46 mg. per gram of cefazolin.

ACTIONS

Microbiology: *In vitro* tests demonstrate that the bactericidal action of cephalosporins results from inhibition of cell wall synthesis. 'Ancef' is active against the following organisms *in vitro:*

Staphylococcus aureus (penicillin-sensitive and penicillin-resistant).

Group A beta-hemolytic streptococci and other strains of streptococci (many strains of enterococci are resistant).

Streptococcus pneumoniae (formerly *D. pneumoniae)*
Escherichia coli
Proteus mirabilis
Klebsiella species
Enterobacter aerogenes
Hemophilus influenzae

Most strains of *Enterobacter cloacae* and indole positive Proteus *(P. vulgaris, P. morgani, P. rettgeri)* are resistant. Methicillin-resistant staphylococci, serratia, pseudomonas, mima, herellea species are almost uniformly resistant to cefazolin.

INDICATIONS

Ancef (sterile cefazolin sodium, SK&F) is indicated in the treatment of the following serious infections due to susceptible organisms:

Respiratory tract infections due to *Streptococcus pneumoniae* (formerly *D. pneumoniae), Klebsiella* species, *Hemophilus influenzae, Staphylococcus aureus* (penicillin-sensitive and penicillin-resistant), and group A beta-hemolytic streptococci.

Injectable benzathine penicillin is considered to be the drug of choice in treatment and prevention of streptococcal infections, including the prophylaxis of rheumatic fever. 'Ancef' is effective in the eradication of streptococci from the nasopharynx, however, data establishing the efficacy of 'Ancef' in the subsequent prevention of rheumatic fever are not available at present.

Genitourinary tract infections due to *Escherichia coli, Proteus mirabilis, Klebsiella* species, and some strains of enterobacter and enterococci.

Skin and soft-tissue infections due to *Staphylococcus aureus* (penicillin-sensitive and penicillin-resistant) and group A beta-hemolytic streptococci and other strains of streptococci.

Bone and joint infections due to *Staphylococcus aureus.*

Septicemia due to *Streptococcus pneumoniae* (formerly *D. pneumoniae), Staphylococcus aureus* (penicillin-sensitive and penicillin-resistant), *Proteus mirabilis, Escherichia coli,* and *Klebsiella* species.

Endocarditis due to *Staphylococcus aureus* (penicillin-sensitive and penicillin-resistant), and group A beta-hemolytic streptococci.

Appropriate culture and susceptibility studies should be performed to determine susceptibility of the causative organism to 'Ancef'.

CONTRAINDICATIONS

Ancef (sterile cefazolin sodium, SK&F) is contraindicated in patients with known allergy to the cephalosporin group of antibiotics.

WARNINGS

BEFORE CEFAZOLIN THERAPY IS INSTITUTED, CAREFUL INQUIRY SHOULD BE MADE CONCERNING PREVIOUS HYPERSENSITIVITY REACTIONS TO CEPHALOSPORINS AND PENICILLIN. CEPHALOSPORIN C DERIVATIVES SHOULD BE GIVEN CAUTIOUSLY IN PENICILLIN-SENSITIVE PATIENTS.

SERIOUS ACUTE HYPERSENSITIVITY REACTIONS MAY REQUIRE EPINEPHRINE AND OTHER EMERGENCY MEASURES.

There is some clinical and laboratory evidence of partial cross-allergenicity of the penicillins and the cephalosporins. Patients have been reported to have had severe reactions (including anaphylaxis) to both drugs.

Any patient who has demonstrated some form of allergy, particularly to drugs, should receive antibiotics cautiously. No exception should be made with regard to 'Ancef'.

Usage in Pregnancy: Safety of this product for use during pregnancy has not been established.

Usage in Infants: Safety for use in prematures and infants under 1 month of age has not been established.

PRECAUTIONS

Prolonged use of cefazolin sodium may result in the overgrowth of nonsusceptible organisms. Careful clinical observation of the patient is essential.

When 'Ancef' is administered to patients with low urinary output because of impaired renal function, lower daily dosage is required (see dosage instructions). A false positive reaction for glucose in the urine of patients on 'Ancef' has occurred with Clinitest® tablets solution.

ADVERSE REACTIONS

The following reactions have been reported.

Hypersensitivity: Drug fever, skin rash, vulvar pruritus, and eosinophilia have occurred.

Blood: Neutropenia, leukopenia, thrombocythemia and positive direct and indirect Coombs tests have occurred.

Hepatic and Renal: Transient rise in SGOT, SGPT, BUN and alkaline phosphatase levels has been observed without clinical evidence of renal or hepatic impairment.

Gastrointestinal: Nausea, anorexia, vomiting, diarrhea, oral candidiasis (oral thrush) have been reported.

Other: Pain at site of injection after intramuscular administration has occurred, some with induration. Phlebitis at site of injection has been noted.

DOSAGE AND ADMINISTRATION

'Ancef' may be administered intramuscularly or intravenously after reconstitution.

Dosage

In adults, usual dosage for pneumococcal pneumonia is 500 mg. every 12 hours. In acute, uncomplicated urinary tract infections, usual dosage is 1 gram every 12 hours. In mild infections caused by susceptible Gram positive cocci, usual dosage is 250 to 500 mg. every 8 hours. In moderate to severe infections, usual dosage is 500 mg. to 1 gram every 6 to 8 hours. 'Ancef' has been administered in dosages of 6 grams per day in serious infections such as endocarditis.

'Ancef' may be used in patients with reduced renal function with the following dosage adjustments: Patients with a creatinine clearance of 55 ml./min. or greater or a serum creatinine of 1.5 mg. % or less can be given full doses. Patients with creatinine clearance rates of 35 to 54 ml./min. or serum creatinine of 1.6 to 3.0 mg. % can also be given full doses, but dosage should be restricted to at least 8-hour intervals. Patients with creatinine clearance rates of 11 to 34 ml./min. or serum creatinine of 3.1 to 4.5 mg.% should be given 1/2 the usual dose every 12 hours. Patients with creatinine clearance rates of 10 ml./min. or less or serum creatinine of 4.6 mg. % or greater should be given 1/2 the usual dose every 18 to 24 hours. All reduced dosage recommendations apply after an initial loading dose appropriate to the severity of the infection.

In children, a total daily dosage of 25 to 50 mg. per kg. (approximately 10 to 20 mg. per pound) of body weight, divided into three or four equal doses, is effective for most mild to moderately severe infections. Total daily dosage may be increased to 100 mg. per kg. (45 mg. per pound) of body weight for severe infections. Since safety for use in premature infants and in infants under one month has not been established, the use of 'Ancef' in these patients is not recommended.

For additional information on dosage and administration, see package literature or contact your SK&F Representative.

HOW SUPPLIED

Ancef® (sterile cefazolin sodium, SK&F)—supplied in vials equivalent to 250 mg., 500 mg., and 1 gram of cefazolin; in Overfill ("piggyback") Vials for intravenous admixture equivalent to 1 gram of cefazolin; and in Pharmacy Bulk Vials equivalent to 5 grams or 10 grams of cefazolin.

SK&F Smith Kline &French Laboratories
Division of SmithKline Corporation, Philadelphia, Pa.

Coagulation changes in eclampsia: Their frequency and pathogenesis

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The maternal coagulation mechanism has been investigated in an effort to identify its role, if any, in the pathogenesis of eclampsia. Thrombocytopenia was identified in 28 of 95 cases (29 per cent), a prolonged thrombin time in 19 of 38 (50 per cent), abnormally elevated serum fibrinogen-fibrin degradation products in two of 65 (3 per cent), and circulating fibrin monomer in one out of 20 (5 per cent). Overt hemolysis was rare (2 per cent). Thus the pattern as well as the degree of change in the maternal coagulation mechanism differed remarkably from that typical of severe abruptio placentae and of prolonged retention of a dead fetus, the classic obstetric models of fast and slow disseminated intravascular coagulation. It is concluded that the coagulation changes when present in eclampsia are effect rather than cause. Moreover, the changes may evolve primarily from platelet adherence at sites of vascular endothelial damage as the consequence of segmental vasospasm and vasodilatation rather than be triggered by the escape of thromboplastin from the placenta into the maternal circulation.

DISSEMINATED intravascular coagulation, characterized by variable degrees of thrombocytopenia, prolongation of the thrombin time, elevated levels of fibrin degradation products in serum, fibrin monomer in plasma, and hypofibrinogenemia, has been described in a great variety of diseases. At times the disseminated intravascular coagulation has been accompanied by microangiopathic hemolysis with deformities of circulating red cells and evidence of acute hemolysis including reticulocytosis, elevated indirect plasma bilirubin, nucleated red cells in the peripheral blood, and, in florid cases, gross hemoglobinemia and hemoglobinuria. The trauma to red cells that leads to their deformity and destruction has commonly been attributed to fibrin strands that formed intravascularly.

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A similar spectrum of hematologic abnormalities has long been recognized in some cases of pre-eclampsia and more commonly eclampsia.[1–4] Moreover, fibrin deposited intravascularly has been described in fatal cases.[5] It is not surprising therefore that disseminated intravascular coagulation has been implicated by some authors in the pathogenesis of pre-eclampsia-eclampsia.[6–10] One more recent theory especially holds that thromboplastin of placental origin invades the maternal circulation at varying rates to produce either "slow disseminated intravascular coagulation" and in turn pre-eclampsia, or "fast disseminated intravascular coagulation" and the pathophysiologic changes of eclampsia.[10]

Reported now are studies carried out over the past two decades on women with eclampsia at Parkland Memorial Hospital to identify more precisely the frequency, the intensity, and, in turn, the pathogenetic significance of those hematologic changes that are implicated especially in disseminated intravascular coagulation.

Materials and methods

Eclampsia was diagnosed when convulsions were documented in an overtly hypertensive woman who was pregnant or had been delivered during the

Table I. Measurements of coagulation factors that imply disseminated intravascular coagulation

	Healthy intrapartum primigravidas	*Most abnormal value for each case of eclampsia*
Platelets*		
Mean, (cu. mm.)	278,000	202,000
−2 standard deviations	150,000	—
<150,000	0/20	28/95
<100,000	0/20	16/95
< 50,000	0/20	3/95
Serum fibrin degradation products†:		
8 μg/ml or less	17/20	57/65
16 μg/ml	3/20	6/65
>16 μg/ml	0/20	2/65
Plasma fibrinogen*		
Mean, mg./100 ml.	415	412
−2 standard deviations	285	—
<285 mg./100 ml.	0/20	7/92
Fibrin monomer		
Positive	1/20	1/20
Plasma thrombin time†		
Mean, (sec.)	8.9	11.3
>2.0 sec. slower than healthy pregnant	—	19/38

*Lowest value identified for each case of eclampsia.
†Highest value identified for each case of eclampsia.

previous 24 hours. In most cases a lengthy period of observation subsequent to delivery has served to rule out other causes for the convulsions. The population studied has been described elsewhere.[11]

Platelet counts were made with standard techniques. The great majority of the counts were made by two individuals.

Fibrinogen-fibrin degradation products were measured with the tanned red cell hemagglutination-inhibition technique of Mersky and associates,[12] the Thrombo-Wellcotest immunologic particle agglutination technique (obtained from Burroughs Wellcome, Inc.), or both. The plasma was promptly separated from 4.5 ml. of whole blood collected in 0.5 ml. of 3.8 per cent sodium citrate solution to which was added 0.05 ml. of Trasylol solution containing 500 kallikrein inactivator units to inhibit fibrinogenolysis. Most often the separated plasma was promptly frozen and assayed at a later time. To measure fibrinogen-fibrin degradation products, 100 units of thrombin were added to 1.5 ml. of freshly-thawed plasma; the mixture was incubated at 37° C. for at least 15 minutes and centrifuged briskly to yield serum.

To identify fibrin monomer the plasma paracoagulation test with protamine sulfate was performed exactly as described by Kidder and associates[13] on plasma collected as described above. A negative test is considered by its developers to be strong evidence against intravascular clotting.

The thrombin time was measured at 37° C. with a BBL fibrometer after mixing 0.2 ml. of bovine thrombin appropriately diluted with saline with 0.1 ml. of fresh plasma collected in citrate-Trasylol. The time for a standard reference plasma (Ci-Trol Normal from Dade Division, American Hospital Supply Corp.) to clot was simultaneously measured with the same diluted thrombin solution. The standardized plasma consistently gave the same value when different lots were compared. Plasma freshly collected from healthy nonpregnant women and from healthy intrapartum primigravid women clotted on the average 1 second faster than did the commercially obtained standardized plasma when a thrombin solution was used that caused the latter to clot in 10 seconds.

Fibrinogen in plasma collected in citrate-Trasylol was measured as thrombin-clottable protein by the method of Ratnoff and Menzies.[14]

Plasma from each specimen of citrate-Trasylol blood was carefully inspected for hemoglobinemia. Free hemoglobin added to normal plasma in a concentration greater than 25 mg. per 100 ml. could be identified. Red cell morphology was evaluated in blood smears stained with Wright's stain.

Each quantitative hematologic measurement was made in duplicate and in practically all of the cases of eclampsia serial measurements were made. The most abnormal value for each case has been used in all of the compilations.

To provide normal pregnancy values, each of the hematologic studies was performed in duplicate on 20 healthy intrapartum primigravid women.

Results

Platelets. For *normal* intrapartum pregnancy the mean value was 278,000 platelets per cubic millimeter; two standard deviations below the mean was 150,000 per cubic millimeter. As shown in Table I, the lowest platelet count with eclampsia was less than 150,000 in 28 of 95 cases (29.5 per cent), less than 100,000 in 16 (16.8 per cent), and less than 50,000 in three (3.2 per cent). The low platelet counts rose to the normal range within 5 days after delivery and frequently then became supranormal.

Serum fibrinogen-fibrin degradation products. Of the 20 healthy intrapartum pregnant primigravidas studied, fibrin degradation products in serum measured 8 μg per milliliter or less in 17, and 16 μg per

Table II. Eclampsia during the second trimester with severe but transient thrombocytopenia the only demonstrated abnormality of coagulation

Date	*Time*	*Platelets (cu. mm.)*	*FDP* (μ/ml.)*	*Fibrin monomer*	*Fibrinogen (mg./100 ml.)*	*Thrombin time† (sec.)*
5/19	16:15	Convulsed				
	18:15	59,000	4	Negative	404	12.0
5/20	07:10	Delivered 750 gram infant after oxytocin induction				
	08:50	73,000	4	Negative		12.0
	10:20		4	Negative	505	11.6
5/21		87,000				
5/22		196,000	Creatinine clearance 91 ml./min.			
5/23		240,000				

*FDP = Fibrinogen-fibrin degradation products.

†Value for normal control plasma = 10.0 sec.

milliliter in three. The maximum values for 65 cases of eclampsia were 8 μg per milliliter or less in 57 and 16 μg in six. Of the two values that exceeded 16 μg per milliliter, one of 128 μg per milliliter was associated with abruptio placentae so extensive as to kill the fetus. The other value of 32 μg per milliliter was obtained with the Thrombo-Wellcotest method whereas an amount less than 8 μg per milliliter was identified by the tanned red cell hemagglutination-inhibition technique. This is the single instance in which a marked difference was found when the two techniques for identifying fibrinogen-fibrin degradation products were compared.

Fibrin monomer. One of 20 plasma samples from the healthy intrapartum primigravidas was questionably positive and one plasma sample from the 20 cases of eclampsia studied was positive for fibrin monomer. Two to six different plasma samples from each of the 20 cases of eclampsia were assayed for fibrin monomer.

Fibrinogen. The mean value for fibrinogen in plasma from the 20 healthy intrapartum primigravidas was 415 mg. per 100 ml. with two standard deviations below the mean of 285 mg. per 100 ml. (Table I). With the use of the lowest measured value for each of 92 women with eclampsia, the mean fibrinogen level was 412 mg. per 100 ml. and in only seven of the 92 was it less than 285 mg. per 100 ml. The three lowest values ranged from 94 to 215 mg. per 100 ml. and each was associated with severe abruptio placentae.

Thrombin time. The thrombin time for plasma specimens from the 20 healthy intrapartum women averaged 8.9 seconds, compared to 11.3 seconds for 38 cases of eclampsia. All but one of the values for eclampsia were above the mean for healthy primigravidas and 19 (50 per cent) were more than 2.0 seconds greater than the mean.

Hemolysis. Overt hemoglobinemia was identified only twice in the 118 cases of eclampsia in which it was carefully looked for. In each instance it promptly cleared after delivery. More common was indirect evidence of hemolysis including elevated indirect bilirubin, reticulocytosis, nucleated red blood cells in the peripheral blood, and mature red cells variably distorted.

Summarized below is a case of antepartum eclampsia with severe hypertension that demonstrates the most common changes in the coagulation mechanism identified during this study.

G. P., a 17-year-old white primigravida at about 24 weeks' gestation, developed headaches and severe epigastric pain. Her blood pressure, taken by her physician just before a grand mal seizure, was 210/110 mm. Hg and the urine protein was 4+. Less than 1 hour later, when she was first seen in the Parkland Obstetrical Emergency Suite, the blood pressure was as high as 194/150 mm. Hg and marked proteinuria was confirmed. Magnesium sulfate intravenously and intramuscularly and hydralazine intravenously were administered according to our standard protocol for eclampsia described elsewhere.[11] Intravenous oxytocin was started and 11 hours later a fetus weighing 750 grams was delivered. The creatinine clearance intrapartum was 67 ml. per minute. Hypertension of decreasing intensity was present for 5 days postpartum; she was normotensive 4 weeks after delivery.

As shown in Table II severe thrombocytopenia and a moderately prolonged thrombin time without other detected abnormalities of coagulation were identified. The platelet count rose to the normal range 2 days after delivery. Evidence for microangiopathic hemolysis was negligible except for a reticulocyte count of 3.6 per cent. Serum bilirubin concentration was less than 1.0 mg. per 100 ml., red cell morphologic changes were minimal, and no nucleated red cells were present in the peripheral blood.

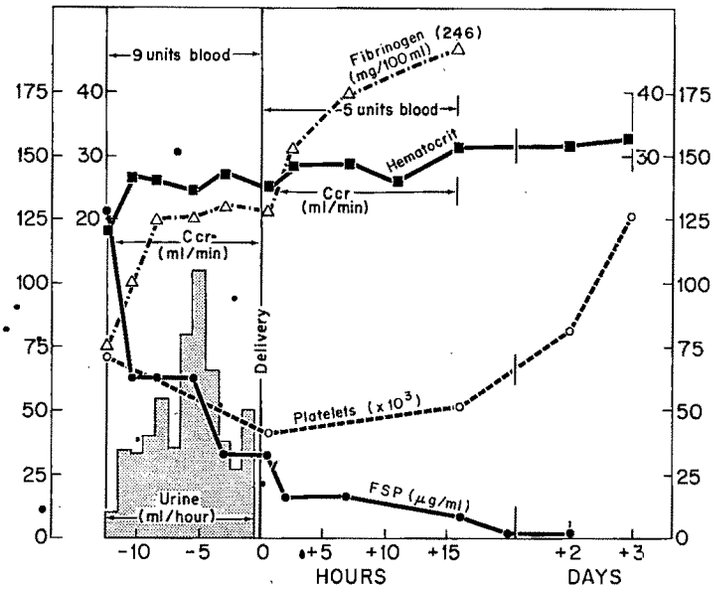


Fig. 1. Serial data from a case of severe abruptio placentae with disseminated intravascular coagulation. None of the classic stigmas of eclampsia developed; hypertension, convulsions, and impaired renal function were absent. *FSP* = fibrinogen-fibrin degradation products.

Comment

There is no question that in some women with eclampsia a variety of hematologic aberrations may develop consistent with those changes ascribed to disseminated intravascular coagulation. The findings reported now, however, do not support the concept that disseminated intravascular coagulation is essential to the development of eclampsia. Against disseminated intravascular coagulation causing the central nervous dysfunction that characterizes eclampsia is the absence in the majority of cases of thrombocytopenia, fibrin monomer in plasma, pathologically increased levels of fibrin degradation products in serum, and hypofibrinogenemia. Moreover, these abnormalities have been identified, albeit very infrequently, in women with pre-eclampsia who did not convulse. Furthermore, in a classic model of disseminated intravascular coagulation induced by thromboplastin entering the maternal circulation, namely, severe abruptio placentae, none of the stigmas of eclampsia, or for that matter pre-eclampsia, need be present.

In our now extensive experience with naturally occurring obstetric models of slow and of fast disseminated intravascular coagulation, the former the consequence of slow escape of thromboplastin into the maternal circulation after fetal death in utero and the latter due to extensive abruptio placentae, pre-eclampsia and eclampsia were not obligatory products of the consumptive coagulopathy. Presented in Fig. 1 are hematologic and renal function data from a case of severe abruptio placentae typical of many that we have studied with profound hypofibrinogenemia, thrombocytopenia, markedly elevated amounts of fibrinogen-fibrin degradation products in serum, and readily identifiable fibrin monomer in plasma. Hypertension, impaired renal function, and convulsions, the classic stigmas of eclampsia, were absent.

When thrombocytopenia did occur in women with eclampsia it was seldom accompanied by either pathologically elevated levels of fibrinogen-fibrin degradation products in serum or of fibrin monomer in plasma and, at times, was the sole abnormality of coagulation identified. Thrombocytopenia in the absence of the other coagulation abnormalities is not compatible with the concept of sudden intense disseminated intravascular coagulation due to thromboplastin leaching from the placenta into the maternal circulation, but implies another mechanism.

Rather than the thrombocytopenia of eclampsia evolving from intravascular coagulation induced by thromboplastin from the placenta, platelet adherence at sites of vascular endothelial discontinuity seems a more likely explanation. Loss of vascular endothelial integrity is known to incite platelet adherence and secondarily fibrin deposition in experimentally induced intense vasoconstriction.[15, 16] Disruption of arterial endothelial lining has been demonstrated in dilated segments of artery that alternate with vasoconstricted segments induced by angiotensin and some other pressor amines. Platelets adherent to the

exposed subendothelial layer and fibrin strands laid down around the platelet aggregates have been clearly identified by scanning electron microscopy.[17] It is postulated, therefore, from the data presented now that in some women with eclampsia comparable endothelial disruption with platelet adherence and fibrin deposition develops which undergoes repair once delivery is accomplished and the vasospasm abates.

The reason for the prolonged thrombin time in half or more of the cases of eclampsia remains unclear, although this abnormality was first described in 1954.[4] At least three mechanisms may be implicated in prolongation of the thrombin time. Two well-described possibilities are very low concentrations of substrate for thrombin, i.e., hypofibrinogenemia, and the anticoagulant effect of large amounts of fibrinogen-fibrin degradation products in the plasma. The laboratory data obtained in the studies reported now do not implicate either of these mechanisms, although the possibility of an unrecognized circulating anticoagulant remains. A third mechanism to account for the prolonged thrombin time is a qualitative alteration in the fibrinogen molecule, sometimes referred to as dysfibrinogenemia.

The concept that eclampsia is caused by disseminated intravascular coagulation engendered by thromboplastin from the placenta entering the maternal circulation implies that treatment with heparin might be beneficial. We are aware of no studies of sufficient magnitude to evaluate adequately any benefits from heparin compared to the obvious risks of such treatment. It is doubtful that heparin would prove highly effective in preventing platelet aggregation at sites of endothelial damage. Moreover, to render the blood hypocoagulable in the presence of severe hypertension and at times serious thrombocytopenia would very likely enhance remarkably the risk of intracranial hemorrhage in women with eclampsia.

All of the cases of eclampsia considered in this report were treated with magnesium sulfate routinely, hydralazine intermittently, and heparin never. The favorable outcomes have been reported in some detail elsewhere.[11]

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Discussion

Dr. Russell Ramon de Alvarez, Philadelphia, Pennsylvania. A little over 20 years ago, when the reports of Drs. Reid, McKay, Pritchard, and others essentially implied that eclampsia and severe preeclampsia produced a coagulation defect, some obstetricians wondered why their investigations of the hypertensive disorders of pregnancy were unable to

uniformly reach the same conclusions stated by some of these authors. What many investigators found, although not new, was confirmation that a hypercoagulable state exists in pre-eclampsia and eclampsia and also that such a state exists in normal pregnancy. This is not to say that the eclamptic patient does not or may not develop coagulation changes just as may any other pregnant patient. Hardly anyone questions that DIC is a reality, but it seems that incidence, pathophysiology and even pathologic significance, specifically in eclampsia, has been exaggerated. In fact, clinical and autopsy evidence of incoagulable states, thromboembolic phenomena, and hemolysis have been and still are found in some, but not all, eclampsia. The same findings may also be found in some noneclamptic pregnant patients as well. As in all clotting, DIC is associated with secondary fibrinolysis, with prolonged prothrombin times, low plasminogen levels, and increased fibrinolysin.

Pronounced coagulation defects may occur in several special situations in pregnancy, such as certain infections, abruptio placentae, sepsis, various hemolytic syndromes, especially uremia and angiopathies, thrombotic thrombocytopenic purpura, and disseminated trophoblastic tumors. Trauma, hemorrhage, and/or the release of bacterial toxins, to which the eclamptic patient is frequently exposed, may activate not only the coagulation process, but the fibrinolytic system as well. As a result, fibrinogen and other coagulation factors are consumed and their concentration reduced; platelets also are decreased. However, the potential of the patient to synthesize new fibrin is tremendous so that fibrinogenopenia, when it occurs, is temporary, where as in thrombocytopenia, the time required for platelet production is prolonged.

Hepatic damage is common in severe pre-eclampsia and eclampsia in our autopsy experience, but Dr. Pritchard has not been afforded this opportunity to note these changes since all his eclamptic patients survived. Even in living eclamptic patients, we have observed definite hepatic impairment in biopsy material obtained percutaneously and at operation in patients with ruptured liver capsule when standard biochemical profiles showed nothing characteristic of extensive or long-standing disease. Fibrinogen may remain quite high with pronounced liver damage, but prothrombin decrease and prolonged prothrombin time are very sensitive indicators of impaired liver function.

A third and not the least explanation is the influence of therapeutic measures such as the administration of blood. Shed blood is required for hemorrhage or to replace blood volume, bank blood, deficient in, if not almost devoid, of platelets and of labile factors necessary to initiate and maintain the coagulation mechanism, is commonly but incorrectly administered.

If shock occurs, another situation exists. This depends upon what caused the shock, however, since the dynamics of septic shock differ from those of hemorrhagic shock or cardiogenic shock. In pregnancy, where cardiac output is high and aggravated by increased peripheral resistance, as in eclampsia, hypoxia, dehydration, and hypovolemia usually play a significant role in initiating a clotting defect. Oxygen extraction in the lung is reduced and what oxygen is extracted is often insufficient to meet the needs created by the hyperpnea and increased cardiac work. This results in further hypoxia and the production of lactic acid accumulation and tissue acidosis.

For years we as obstetricians have featured the kidney, liver, and brain in the pathology of eclampsia, based upon the study of autopsy material. However, ventilatory and circulatory dynamics, with the exception of pulmonary edema, probably have received less attention than they deserve. Studies of these processes should augment rather than replace studies of cardiac, vascular, and renal function in understanding the basic alterations in the hypertensive disorders of pregnancy. The increased work of the heart, atelectasis, pulmonary edema, and pneumonia, which commonly occur in these patients have received the therapeutic attention they deserve and have been responsible for development of inhalation therapy teams, and the providing innovative methods of respiratory assistance and the continuous creation of versatile artificial devices. On the other hand, the overly aggressive use of some of these methods, the use of too high oxygen concentrations and errors in judgment tend to vitiate some of these advances by contributing to the production of harmful coagulation problems.

The alterations of coagulation in eclampsia are probably interrelated and seem to be due to: (1) factors responsible for hypovolemia; (2) factors responsible for shock, electrolyte, and acid-base balance; (3) vascular damage including hypoxia, or some other direct trauma to certain organs (placenta, uterus, vagina, lung, liver, kidney brain); and (4) treatment factors (usually treatment errors resulting from failure to replace the factors necessary in the multiple sequences of the coagulation mechanism).

What seems to be debatable is not so much whether the coagulation problems are coincident to or directly due to eclampsia but whether a lack of full agreement among investigators may result from differences in technology and methodology. From a purely clinical standpoint, we undoubtedly are not studying eclampsia alone but also one or more of the various complications of this major complication of pregnancy. It is obvious that we are dealing with several complex physicochemical, biologic, and clinical issues. In order to attempt to solve and develop meaningful answers to these problems, even though unduly ambitious, I believe it would be helpful to establish a multicentered interdisciplinary program using identical methodology and a uniform

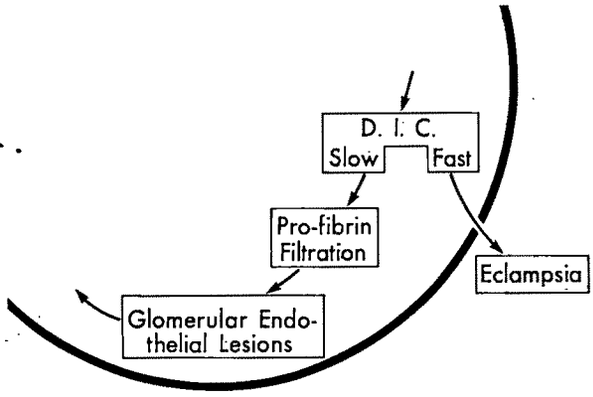


Fig. 1. A possible factor causing the glomerular endothelial lesion. (By permission of J. Obstet. Gynaecol. Br. Commonw.)

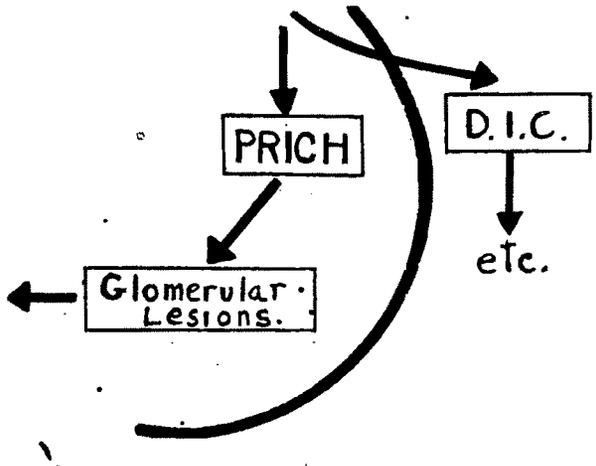


Fig. 2. A revision impelled by Dr. Pritchard's data.

protocol of evaluation of each coagulation factor in eclamptic and control patients for each of the causes of alteration of the coagulation mechanism.

DR. ERNEST W. PAGE, Piscataway, New Jersey. Every now and then some ugly facts arise to slay a beautiful hypothesis. Dr. Pritchard's fine presentation will force me to alter my preconceived concept (always a painful process) regarding the pathogenesis of eclampsia. He refers to my hypothesis in the manuscript; so let me first explain to you what it is or, as of now, was.

Fig. 1 is one of several published in 1972 which postulates that part of an "inner vicious circle"—the most controversial part, perhaps—is a rapid disseminated intravascular coagulation (DIC) which leads to platelet-fibrin thrombi in the brain, liver, and other organs. In adopting this, I was strongly influenced by my colleague in San Francisco, Dr. Donald McKay,[1] an astute pathologist, who wrote this statement in 1972: "In eclampsia, the sudden occlusion of arterioles, capillaries and venules by platelet-fibrin thrombi leads to ischemic tissue damage. . . . When the clotting mechanism is triggered, aggregates of platelets and thrombi are swept into the microcirculation of the brain. . . . If the vessels involved supply a portion of the motor cortex, the ischemia produced results in convulsion. . . . . Lodgement of the platelet aggregates damages the endothelium locally and blood pressure forces red blood cells out into the perivascular region, producing 'ring hemorrhages.' Such hemorrhages are found in all patients with eclampsia and represent the only lesion that occurs virtually 100 per cent of the time."

Now I may have assumed erroneously that what happens in eclamptic patients who die, about whom Dr. McKay knows a great deal, also happens in those who survive, about whom Dr. Pritchard knows a great deal because none of his eclamptic women die. But if Dr. McKay's explanation is incorrect, then what is the cause of the convulsions?

I was also intrigued by the concept that the deposition of some fibrinogen derivative in the endothelial cells of the glomeruli, a phenomenon noted in essentially all patients with pre-eclampsia or eclampsia, is the result of a slow and perhaps intermittent intravascular coagulation. I have produced such lesions by injecting placental thromboplastin intravenously. I am also intrigued by the observations of Wood[2] that serial observations in pre-eclampsia reveal a rise in plasma cryofibrinogen prior to an exacerbation and a rise in fibrin degradation products during remission. Several British observers who report evidence of DIC in some patients with pre-eclampsia observe it only in those with proteinuria. I would like to ask Dr. Pritchard if the 29 per cent who had significant thrombocytopenia also had proteinuria which was more intense.

Before totally capitulating to Dr. Pritchard's obvious conclusion, I would like to raise some additional questions.

The illustration of abruptio placentae as an example of rapid intravascular coagulation could be erroneous. While I may have been the first to promulgate that concept in 1948, a careful study of the monograph by Per Nilsen[3] in 1963 leads me to believe that abruptio placentae is an example of extravascular coagulation with maternal reabsorption of serum.

A major question is whether the parameters measured by Dr. Pritchard are truly sensitive indicators of mild—perhaps episodic—intravascular coagulation. For example, fibrinogen chromatography might reveal abnormal findings in 100 per cent of eclamptic patients. Perhaps we really need measurements of platelet and fibrinogen turnover rates, but these cannot ethically be done because of the radioactivity involved. I am impressed by the following statement from the literature: "Disseminated intravascular coagulation of considerable magnitude may be present without gross changes in the fibrinogen concentration. . . . We

need data on fibrinogen turnover rates in this condition."[4]

Despite these reservations, I will accept the author's conclusion that DIC does not occur in all eclamptic patients. It becomes necessary for me to remove DIC from the inner circle of pathogenesis (Fig. 2) and substitute an unknown, some protein-rich immunologically confusing happenstance (abbreviated as PRICH). No one is better equipped to elucidate the nature of PRICH than Dr. Pritchard.

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DR. FRITZ K. BELLER, Muenster, Germany. Dr. Pritchard has again presented data of an unusual approach of clinical investigation. He is respected for choosing a small segment of a controversial issue applying clear methodology and attempt to clarify the issue. His conclusions have on occasion changed our clinical management. On other occasions the new approach has stimulated thinking even if one did not accept the concept of the author. On a few occasions Dr. Pritchard has re-evaluated previous conclusions. The present paper is such an example.

Dr. Pritchard and his co-workers presented data to indicate that coagulation changes fail to be present in eclampsia. Based on their results they dismiss the concept that "fast" disseminated intravascular coagulation (DIC) is a pathophysiologic event in eclampsia.

I would entirely agree with him and so would many of our collegues working in this field, if the authors were discussing pre-eclampsia rather than eclampsia. Many of us were puzzled by the observation that in pre-eclamptic patients the only coagulation change is a rather infrequent thrombocytopenia. An increase in fibrin(ogen) breakdown products is controversial. We have completed a recent study using the TRCH II method according to Merskey, which was also used by the authors. Our data, however, indicated an increase in fibrinogen breakdown products in patients with pre-eclampsia.[1]

In contrast to pre-eclampsia most authors observed in patients developing eclamptic seizures, in contrast to Dr. Pritchard, coagulation changes consistent with the concept of an activation of the coagulation system. By reviewing the data of Galton and his co-workers,[2] who investigated more than 150 patients with toxemia of pregnancy in Mexico City, we observed only an infrequent thrombocytopenia in severe pre-eclamptic patients. In four out of seven patients with eclampsia, however, thrombocytopenia was present together with low plasma fibrinogen values and other coagulation abnormalities to indicate DIC. More sophisticated techniques were not available at that time. McKay[3] has recently pointed out that patients with eclamptic seizures developed spherocytosis and helmet cells, an abnormality of red blood cells. Many years ago Brian and associates[4] demonstrated the relationship between this red cell abnormality and fibrin deposition in the microcirculation. This well recognized work resulted in the terminolgy of microangiopathic hemolytic anemia, which should not be confused with the hemolytic uremic syndrome. Dr. Pritchard has focused attention on this symptomatology on previous occasions.

In extensive animal experimentation we have confirmed not only the relationship between spherocytosis and DIC but also the relationship between an increase in plasma hemoglobin concentration and DIC.[5–7]

Based on the discussion of the relationship between spherocytosis and DIC I would assume that the authors would fail to find glomerular fibrin deposition in their material. Fibrin deposition in the glomerular capillary, the hallmark of the generalized Shwartzman phenomenon, was observed by McKay and associates[8] in patients with eclampsia. Coagulation data and morphologic observations seem to fit nicely together to indicate DIC as a pathophysiologic event in eclampsia until the present observation by Dr. Pritchard.

Why may it be that patients are much better off in Dallas than they are in San Francisco or elsewhere? May it be that the improvement in the therapy for pre-eclampsia and eclampsia has changed the pathophysiologic events? This seems to be a likely explanation, since one paper regarding modern treatment of toxemia of pregnancy is originating from the Dallas group. Or may it be that we are using the terms eclampsia and pre-eclampsia but meaning different things? It is our feeling at present that toxemia of pregnancy is a rather large *omnium gatherum* of disease entities. Toxemia of pregnancy is based on symptomatology. Renal biopsies have demonstrated that the morphology may either be the genuine lesion of pre-eclampsia or renal disease as, for instance, glomerulonephritis. For these thoughts I would appreciate the comments of Dr. Pritchard in order to offer a possible explanation for his results differing from many groups who all worked with accepted methodogical standards.

The concept of the authors of endothelial disruption with platelet adherens and secondary fibrin incorporation based on vasoconstriction is challenging. I always had difficulties in understanding the mechanism for the release of placental tissue thromboplastin in the maternal circulation to indicate DIC. This concept is merely based on the observation that DIC can be produced in animals by injecting tissue thromboplastin. There are a variety of additional trigger mechanisms of the blood coagulation system. Antigen-antibody complex formation triggers as nicely, or

endotoxin, to name only a few. The organism can obviously react to the activation of the coagulation system by only a very limited pathway. I believe this is what is meant by Dr. McKay's[9] expression for DIC as an "intermediary mechanism of disease." Otherwise it would be unexplainable to observe identical morphology in such a wide range of diseases. DIC has to be considered as a complicating feature and not an etiologic event. More sophisticated techniques as, for instance, gel filtration for the demonstration of fibrin monomers of $\mu = \mu$ dimmertyp may support the concept of Dr. Pritchard.

Regarding the treatment, I find myself in complete agreement with Dr. Pritchard's objection to using heparin. I can hardly see any coagulation assay for the evaluation of beneficial effects of the heparin treatment in toxemia of pregnancy. In eclampsia heparin should be contraindicated. Again it was McKay who focused our attention on the fact that eclamptic seizures are associated with intracerebral bleeding. Heparin should therefore not be used as it is not used in any other form of cerebral vascular accident.

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DR. DENIS CAVANAGH, St. Louis, Missouri (by invitation). I congratulate Dr. Pritchard for de-emphasizing the place of DIC in eclamptogenic toxemia, and I hope that we will be seeing less treatment with heparin without good scientific basis. We, like Dr. Pritchard, while impressed with the importance of DIC in abruptio placentae, have not been able to demonstrate it to any significant degree in eclamptogenic toxemia in our patients. Also, in our studies of baboon toxemia models, published last year,[1] although we were able to demonstrate hypertension, proteinuria, and even deposition of fibrinoid material in the kidney, despite exhaustive and repeated coagulation profiles on our baboons, we were unable to show any evidence of DIC. I must say I was very disappointed with these findings, but now Dr. Pritchard has convinced me that perhaps the baboon toxemia model is not as defective as we thought it was with regard to the coagulation changes.

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DR. MICHAEL J. JORDAN, New York, New York. In the late thirties, Arnold and Fay at Temple University documented the bizarre changes in the brain and spinal cord of patients autopsied following eclamptic convulsions. These bizarre changes were attributed to trauma and increased intracranial pressure and many of patients had what was called "hidden edema of the brain and spinal cord associated with thromboembolic phenomenon." The fact that these changes were present in the brain and spinal cord is of particular importance and indicates the necessity of a closer clinical pathological study. To me Dr. Pritchard's excellent presentation raises the question that perhaps the use of mini-heparinization in these patients may be indicated and not dangerous at all. I would appreciate hearing Dr. Prichard's thoughts.

DR. PRITCHARD (Closing). In regard to the comment that mini-heparin may not be dangerous, I would like to speak out emphatically that as a general policy clinicians ought to use drugs and techniques only when sure that they do good. In clinical practice we should not have to prove that something is not dangerous. I personally believe that heparinization is dangerous in several circumstances, including that of the hypertensive, thrombocytopenic woman. I would certainly welcome a study that would clarify whether heparin is or is not of benefit. We have used a standard treatment regimen for eclampsia for some time which certainly did not include heparin. The results are in press and will be out in October. They provide a standard against which someone can compare other treatments, including heparin.

In regard to Dr. Beller's comments on evidence of coagulopathy in four of seven cases of eclampsia, in my residency days (and nights) we had three cases of eclampsia, all seen within two months, and all demonstrating severe coagulopathy, intense hemoglobinemia, and hemoglobinuria. In the next 20 years and 160 cases I have seen one more; so, small samples

can lead to incorrect answers. Certainly, changes consistent with DIC may occur with eclampsia, and especially in those circumstances in which there is also some degree of abruptio placentae (which occurred in about 7 or 8 per cent of our cases of eclampsia). The fact that in Dallas we see women who convulse but we quite soon treat them and deliver them promptly further supports my contention that the coagulopathic changes are the effect of the disease rather than pathogenetically causing the symptomatology and physical findings of eclampsia.

We have done very few renal biopsies for one reason: Morbidity can be significant under these circumstances.

Dr. Lindheimer and associates from the University of Chicago have recently presented data which demonstrate that the renal lesion of fibrin deposited in and around the endothelium of the glomerular capillaries is not an early development. They have studied 31 patients who, to their satisfaction, had mild to moderate pre-eclampsia, but found fibrin by immunofluorescence in only seven, as I recall (certainly less than half of those studied), and in each instance the amount of fibrin was extremely small, being rated as somewhere between ± and 1+. They concluded that the deposition of fibrin in the kidney is the effect of the disease and not the cause of the disease.

I was intrigued by Dr. Page's comments in regard to abruptio placentae. I grew up following his concept that the coagulation defects of abruptio placentae are the consequence of thromboplastin from the placenta and perhaps decidua entering the maternal circulation and I set out to disprove it. I accumulated data which at one point in time I interpreted in the same way as did Nilssen, i.e., the coagulopathy was localized in the uterus. Dr. Page abandoned his concept in view of Nilssen's findings. I abandoned our concept of coagulopathy confined to the uterus in view of our subsequent findings that, while there is a lot of fibrin deposited within the uterine cavity, not enough fibrin is there to account for all that is missing. More important, I think, is recognition that one characteristic feature of intense coagulation is the production of fibrin degradation products. If fibrin deposition was occurring just in the uterus, the level of fibrin degradation products ought to be higher in the uterus than in the intravascular compartment since those in plasma would represent leaching from the uterus into the circulation. In our experience, within the limit of measurement, fibrin degradation products in the uterus and in the maternal circulation are of the same order of magnitude. Therefore, I believe there is appreciable DIC with abruptio placentae rather than just focal intrauterine coagulation.

In regard to Dr. de Alvarez's opening comments and the diversity of the findings of disseminated intravascular coagulation, I conclude with the following:

DIC (Disseminated Intravascular Coagulation)
See DIC
See DIC run and play in the blood vessels
See Jane
See Jane run and play
See Jane not run but play and get pregnant
Jane will now get DIC since
DIC occurs in pre-eclampsia–eclampsia
DIC occurs in abruptio placentae
DIC occurs with most obstetric complications
If Jane's baby Dick is asphyxiated, Dick may get DIC
Jane should not have gotten pregnant
But the pill, while preventing Dick, may cause DIC
But so can malaria, virus infections, a bad bump on the head, even infectious mononucleosis
More and more it appears most things in life can cause DIC
Probably no one ever dies alone
There is always DIC

Diagnostic ultrasound for detection of intrauterine growth retardation

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There were 738 fetal BPD determined in 468 normal obstetric patients between 16 and 43 weeks in whom (1) the size of the uterus on initial examination corresponded to the duration of amenorrhea ± 1 week and (2) there were no complications during the pregnancy. The mean BPD ± 2 S.D. was determined for each week. The rate of BPD growth was found to be 0.26 cm. per week from 18 to 38 weeks. One hundred random high-risk obstetric patients in whom the size of the uterus on initial examination corresponded to the weeks of amenorrhea ± 1 week were studied. Two patterns of suspected IUGR are observed: one shows BPD values more than 2 S.D. below the mean; the other manifests a decreased Δ BPD. Combinations of the two may be seen. At delivery seven neonates were identified who were SGA and could not be detected in utero by single BPD measurements.

INTRAUTERINE growth retardation has a severely adverse affect on the outcome of pregnancy. The neonate has an increased incidence of meconium aspiration, asphyxia, acidosis, polycythemia, hypoglycemia, and impaired growth and development. Although these problems are manifest in the nursery, the problem really begins in utero. Morbidity and mortality rates can be reduced by accurate prenatal diagnosis of impaired fetal growth, appropriately timed delivery, and intensive intrapartum and neonatal care.

If preterm delivery is to be used, the detection of intrauterine growth retardation (IUGR) must be sufficiently accurate to detect most of the growth-retarded fetuses but avoid the possibility of early delivery of an unaffected fetus. This study evaluates the use of diagnostic ultrasound fetal biparietal diameters (BPD) to detect IUGR.

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Presented by invitation at the Eighty-sixth Annual Meeting of the American Association of Obstetricians and Gynecologists, Hot Springs, Virginia, September 4-6, 1975.

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Material and methods

A total of 738 fetal biaparietal diameters (BPD) were determined in 468 normal obstetric patients between 17 and 43 weeks' gestation. The patients were included in this study only if (1) the size of the uterus on initial examination corresponded to the last normal menstrual period ± 1 week and (2) there were no complications associated with the pregnancy. The mean BPD ± 2 S.D. was determined for each week of gestation.

The ultrasound scan was performed with the B-mode of a Unirad Sonograf II instrument utilizing a 2.5 MHz transducer. Three Polaroid pictures of the fetal head were taken with the falx cerebri centrally located (Fig. 1). A centimeter scale was superimposed on each picture. Scans in which the falx cerebri were not identified were excluded from the study. The fetal head was measured with calipers from the outer aspect of the head to the inner aspect of the contralateral side. The average of the three measurements was used as the biparietal diameter. Each reading was performed by the same investigator. The accuracy and reproduci-

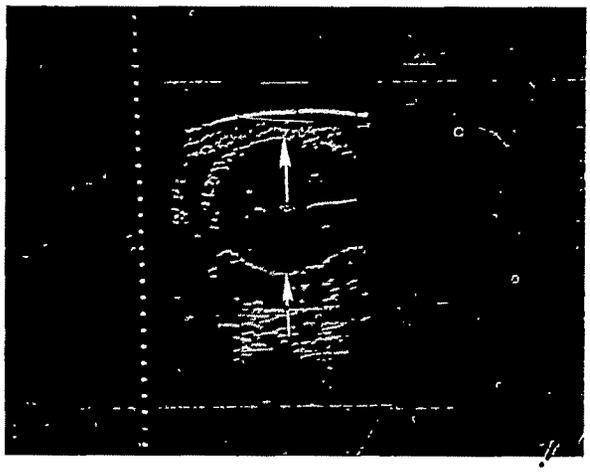


Fig. 1. Biparietal diameter of the fetal head with the falx cerebri centrally located.

Table I. Composition of high-risk group

	No.
Pre-eclampsia	24
Chronic hypertension	18
Previous infant <6 lb.	13
Rh-immunized	10
Anemia <10 Gm.	8
Suspected IUGR	6
Under 16 years of age	5
Heart disease	5
Diabetes	3
Vaginal bleeding	3
Weight <100 lb.	2
Other conditions	14

bility of B-mode ultrasound BPD has been confirmed.[1–6] The error of the method was found to be 0 to 3 mm.[1–6]

In the same population, the normal BPD growth curve was analyzed by the method of least squares. The rate of growth of the BPD (Δ BPD) was determined. The rates were analyzed for both 3 and 4 week intervals and the standard deviation was computed. With standard normal distribution tables, a threshold value was calculated on the basis of 10 per cent of the population having a Δ BPD below the threshold. The 10 per cent figure was used because this corresponded to the definition of SGA.

One hundred random high-risk obstetric patients whose uterine size on the initial visit corresponded to the last normal menstrual period (± 1 week) were studied. Patients with multiple gestations were excluded. The fetal BPD was determined on one or more occasions after the sixteenth week of gestation. In addition, the rate of growth was determined by the Δ BPD for 3 week and 4 week periods.

The babies resulting from these 100 high-risk pregnancies were evaluated by clinical estimates of gestational age according to the parameters of Lubchenco.[7] Height, weight, and head circumferences were plotted on Colorado growth charts. Neonates whose birth weight fell below the tenth percentile were designated as small for gestational age (SGA). Fetuses whose BPD at any time fell below 2 S.D. on the normal curve were categorized as suspected IUGR.

Results

The mean values ± 2 S.D. from the 468 patients having 738 BPD at various weeks of gestation are shown in Fig. 2.[6] The mean values show progressive increase with each week of gestation.

The Δ BPD of this population was determined. The BPD growth curve from 18 to 38 weeks' gestation revealed a straight line ($r = 0.96$, $p < 0.001$). The slope of the line is 0.260 (±0.005) cm. per week, indicating that normal growth per week is 0.26 cm. any time from 18 to 38 weeks' gestation.

The mean rate of growth for these patients where Δ BPD was measured over a 3 week interval was 0.78 cm ($n = 21$), S.D. = 0.32. Using tables for the area under a standard normal curve, this translates to a threshold of 0.4 cm. for a 3 week period below which 10 per cent of the patient population should fall. The mean Δ BPD for the 4 week interval was 1.01 ($n = 47$) S.D. = 0.34. This translates to a threshold of 0.6 cm. for a 4 week period below which 10 per cent of the patient population should fall. In this study, those patients with a Δ BPD under 0.4 cm. for 3 weeks or 0.6 cm. for 4 weeks will be considered to have IUGR.

A total of 240 BPD determinations were performed in the 100 randomized high-risk obstetric patients. The composition of this study group is shown in Table I. The 100 patients had 111 pre-existing conditions or complications of pregnancy. Such conditions as epilepsy, history of prematurity, and excessive weight gain were included in "other conditions."

The individual values are plotted as a scattergram on the normal BPD curve in Fig. 3: 183 determinations fell within the mean ± 2 S.D.; 20 determinations were above and 37 were below 2 S.D.

BPD values. Sixteen patients had serial BPD's with at least one value falling below 2 S.D. Of these, seven patients had SGA infants at birth (Fig. 4). Nine had AGA infants at birth (Fig. 5). The birthweight percentile ranged from the eleventh through the thirty-seventh percentiles with an average at the eighteenth percentile.

Fig. 6 shows seven patients whose infants were evaluated as SGA at birth but whose single BPD's were within the normal range.

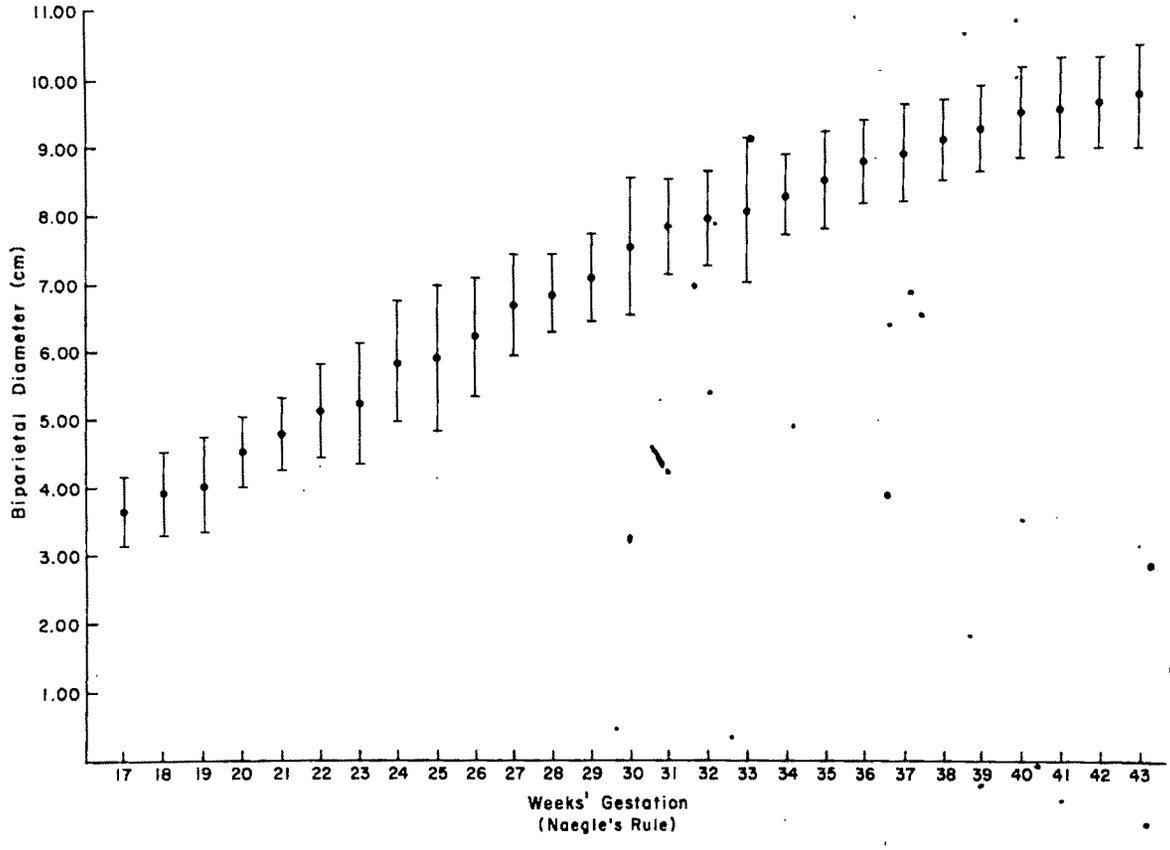


Fig. 2. Mean biparietal diameter ±2 S.D. from 17 to 43 weeks' gestation.

Table II. SGA infants delivered by 100 patients

BPD	*No.*
16 Low	7 SGA
	9 AGA
7 Normal	7 SGA
2 Low (Δ)	2 SGA

Δ BPD. Two patients delivering SGA infants had serial BPD values which were within normal limits but the rate of increase was arrested. These are shown in Fig. 7. Patient A had a Δ BPD for 3 weeks of 0.3 cm. and patient B had a Δ BPD for 4 weeks of 0.5 cm. Both of these patients had chronic hypertension. Patient X and patient Y in Fig. 4 had decreased Δ BPD in addition to low BPD. Patient X had severe diabetes with hypertension and patient Y had rheumatic heart disease with aortic and mitral insufficiency.

Of the 16 SGA infants, nine were diagnosed prenatally by low BPD or arrested Δ BPD (Table II). Nine patients with low BPD delivered AGA infants. The seven patients with normal BPD who delivered SGA infants had only single BPD determinations. Two patients had low Δ BPD with SGA infants. The conditions associated with SGA infants are listed in Table III.

Table III. Conditions associated with SGA infants

	No. of SGA infants	*Detected by ultrasound*
Hypertension	6	4
Suspected IUGR	2	2
Pre-eclampsia	2	0
Diabetes	1	1
Heart disease	1	1
Excessive weight gain	1	1
Epilepsy	1	0
Third-trimester bleed	1	0
Previous small baby	1	0

Comment

IUGR is associated with an increased perinatal mortality rate and if the baby survives it causes a host of morbid conditions. Prenatal diagnosis gives the opportunity to decrease these mortality and morbidity rates. In the study of Mann and associates,[8] four of the 33 fetuses in which the diagnosis could not be made

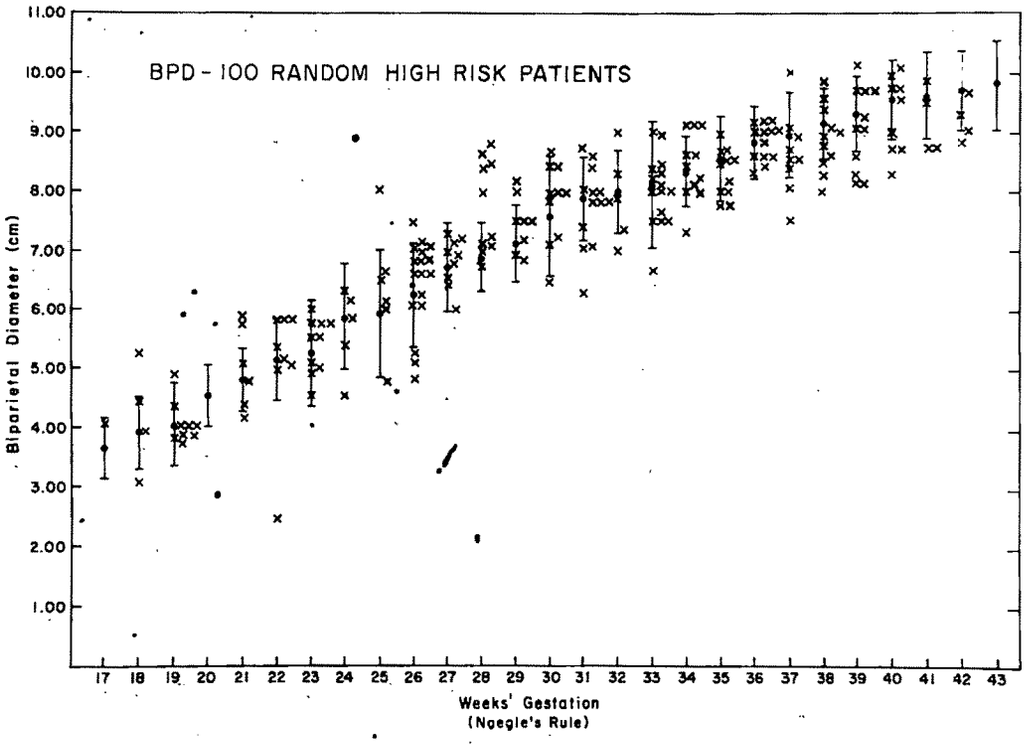


Fig. 3. Scattergram of biparietal diameters from 17 to 43 weeks' gestation in 100 random high-risk pregnancies.

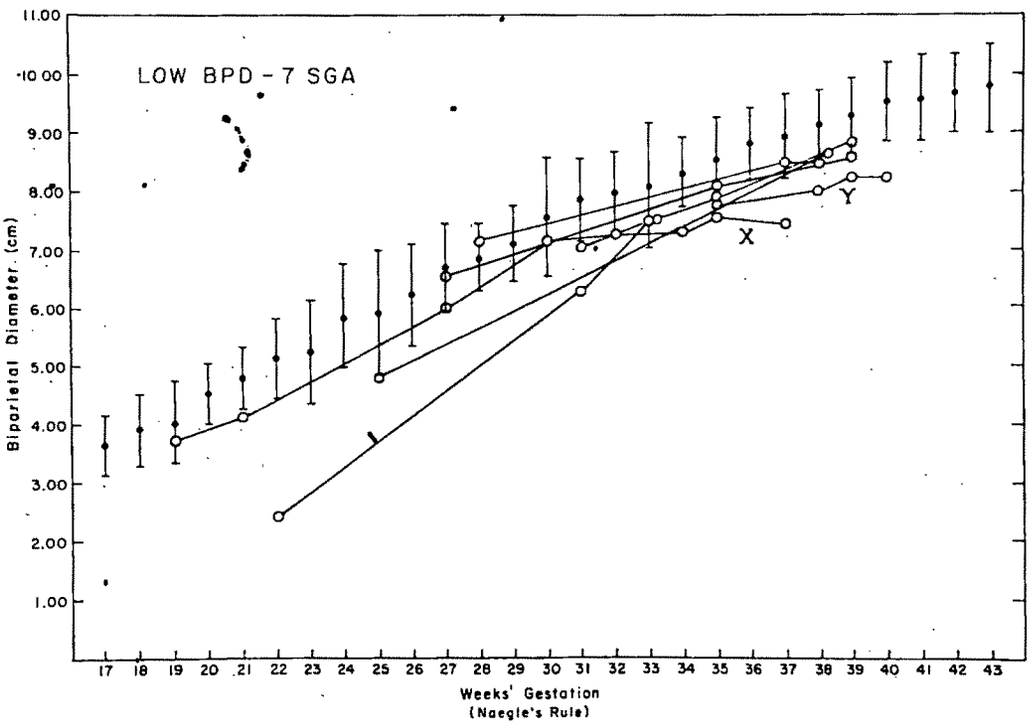


Fig. 4. Serial biparietal diameters from patients with at least one value greater than 2 S.D. below the mean, delivering SGA infants.

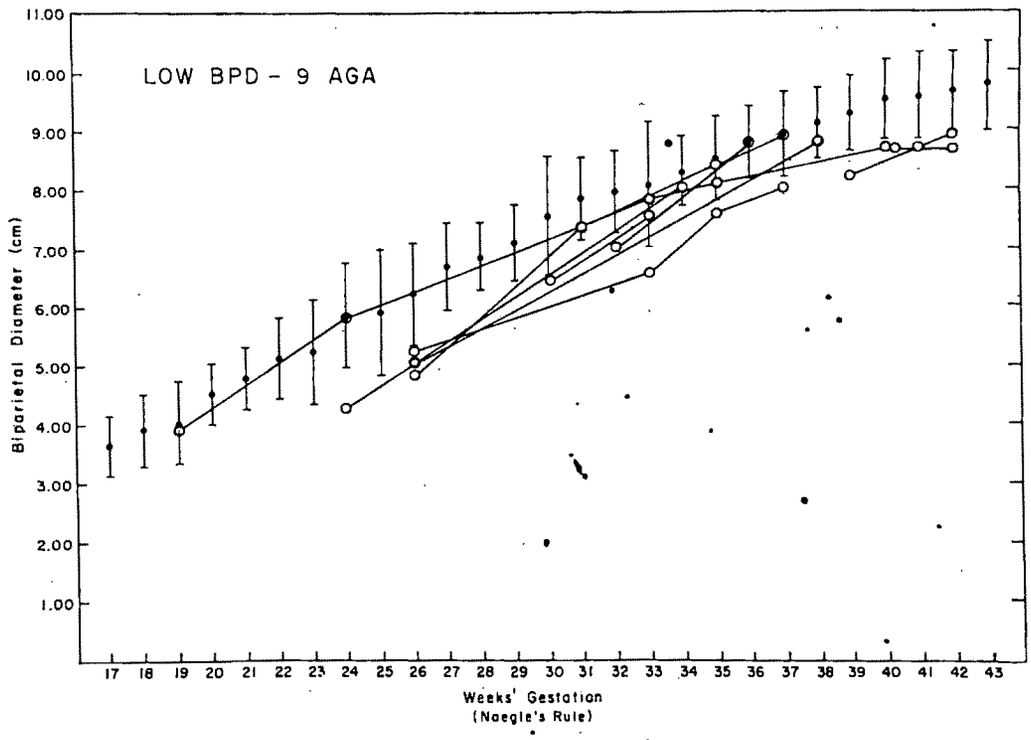


Fig. 5. Serial biparietal diameters from patients with at least one value greater than 2 S.D. below the mean, delivering infants that were AGA.

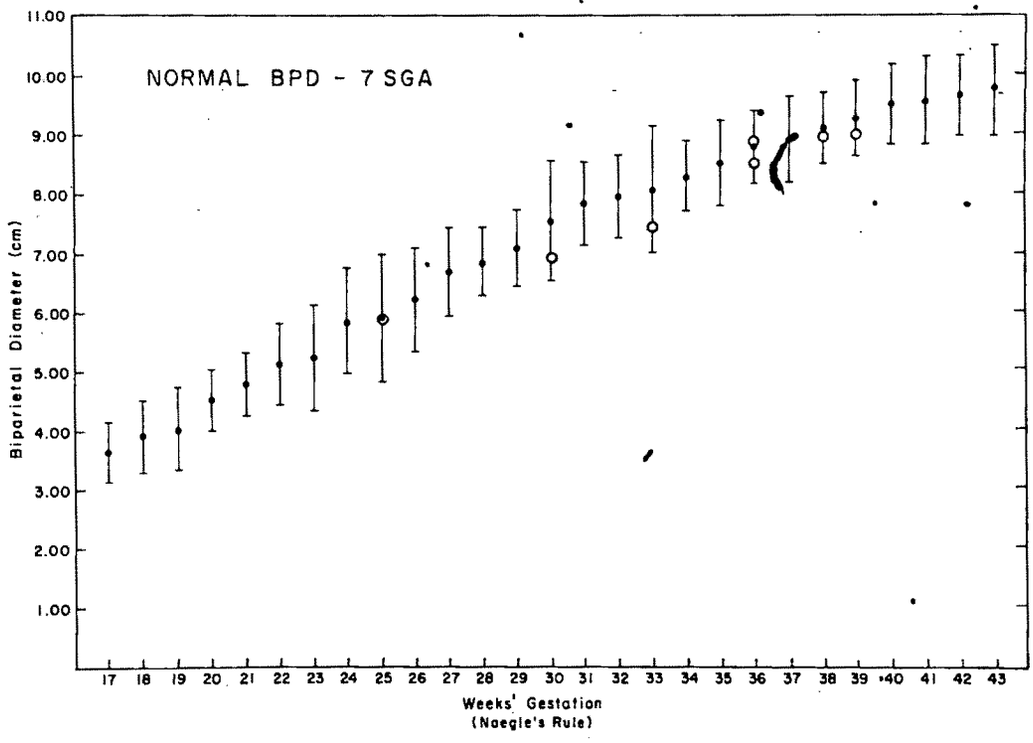


Fig. 6. Single biparietal diameter determinations within the normal range from patients who delivered SGA infants.

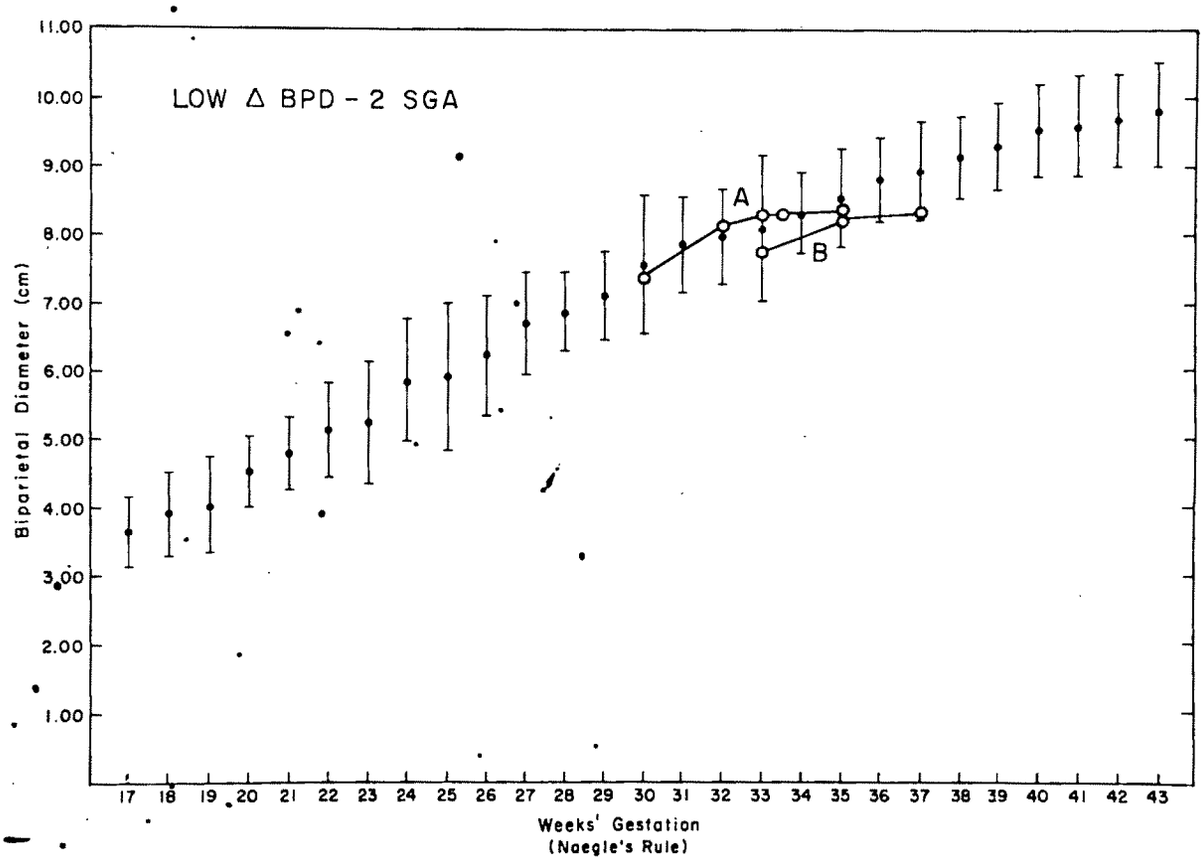


Fig. 7. Two serial biparietal diameters within the normal range which demonstrate an arrested Δ BPD. Both infants were SGA.

prenatally died between 38 and 40 weeks, whereas all 12 fetuses diagnosed prenatally survived.

The accuracy and reproducibility of the ultrasound fetal BPD is well established. The difficulty in detecting IUGR is due to the variations in normal growth of fetal BPD. The range of normal is relatively large for each week of gestation. Therefore, a single value is seldom helpful.

Our statistical evaluation indicates that the BPD growth curve from 18 to 38 weeks' gestation is a straight line. Willocks and associates[9] found that the BPD growth curve was "approximately in a straight line except for the last few weeks of pregnancy." These findings differ from the report of Murata and Martin[10] who, on 105 determinations on 75 patients, stated that the BPD increased more rapidly between 24 and 28 weeks than afterward.

The error of the BPD measurement method is 0 to 3 mm. The Δ BPD is 0.260 (±0.005) cm. per week. Therefore, theoretically, the least growth should be 0.255 per week. Campbell[11] stated, "It is possible to make assessments of the fetal growth rate from two measurements separated by 1 week but there is no doubt that the more numerous the measurements and the earlier in pregnancy they are started, the more accurate and useful will be the diagnostic predictions." Willocks and associates[9] found that 70.4 per cent of SGA babies had a growth rate of less than 0.17 cm. a week. To be clinically useful, the anticipated growth must be greater than the maximum error of the method. In order to minimize the effect of measurement error, we believe the Δ BPD should be applied on the basis of 3 or 4 week intervals.

It appears that there are two ways to detect IUGR by ultrasound BPD determinations. One is to observe BPD values more than 2 S.D. below the mean. The other method is to observe serial values in the normal range which arrest their rate of increase as determined by the Δ BPD. We suggest that the former detects IUGR as seen with such factors as constitutional, malnutrition, and congenital infection, whereas the latter is seen with vascular disease. Of course, a combination of the two may be seen.

In this study, nine of 16 SGA infants were diagnosed prenatally. It appears that the rate of detection by ultrasound alone is 50 to 60 per cent. Since a significant

percentage of growth-retarded fetuses go undetected with this method, we must continue to seek additional methods of detection.

We wish to thank Kay Scott of the Health Sciences Computer Center for her statistical analysis.

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Discussion

DR. HENRY A. THIEDE, Rochester, New York (by invitation). Dr. Queenan's manuscript is enjoyable to read. He displays his data in a lucid and succinct manner that is characteristic of his orderly approach to research.

The importance of having a safe and reliable means for detecting intrauterine growth retardation certainly cannot be overemphasized. However, the ability to detect only 50 to 60 per cent of small-for-gestational-age (SGA) infants by biparietal sonography is disappointing but not really surprising. In essence the authors have nicely demonstrated by fetal sonography exactly what might have been conjectured on the basis of existing clinical information.

Dr. Queenan and his associates have appropriately started by calculating the biparietal diameter (BPD) scale for their particular laboratory. The details for this are not provided in this paper, but several questions do come to mind concerning the methods. For example, do you think you might have sharpened your estimates of gestational age, used in calculating the normal growth curves, by including in your criteria a clinical assessment of the neonates? Gestational age based on menstrual history is accurate only 80 per cent of the time[1] and fundal height measurement is at best only modestly reliable as an approximate indicator of gestational age.[2]

Another question relates to the use of the tenth percentile of the Lubchenco parameters for selecting the SGA babies. The altitude in Denver is 5,280 feet, whereas the altitude in Louisville is 450 feet. This would seem to be roughly equivalent to using the third percentile of Usher and McLean's[3] standards obtained at sea level. Could this account for your inadvertently eliminating a significant number of SGA babies from your series?

Dr. Charles Hohler, Director of the Rochester Regional Perinatal Center Ultrasound Laboratory, has recently reviewed the last 1,500 patients on whom he has performed fetal ultrasonic cephalometry. Serial biparietal diameters were done on 311 patients; single biparietal diameter determinations were performed on the remaining 1,189 patients. These 1,500 patients produced 43 (3 per cent) clinically confirmed SGA babies.

Single fetal biparietal diameters were obtained on 24 of these SGA babies and 13 (54 per cent) had measurements below the tenth percentile (Table I).

Serial fetal biparietal diameters were obtained on the remaining 19 of the SGA babies. Ten (53 per cent) had Δ BPD's less than half of mean; in 12 (63 per cent) the last measurement was below the 10th percentile; six (32 per cent) had both a low BPD and a poor Δ BPD.

Over all, 23 (53 per cent) of the 43 SGA babies were correctly predicted by ultrasonic cephalometry. These results are quite comparable to those of the authors.

Statistically speaking, the Δ BPD as a screening test for IUGR has a false-negative rate that is quite good (3 per cent) but the false-positive rate is far too high (44 per cent); the specificity is good (3 per cent) but the sensitivity is poor (53 per cent). This suggests that the test would be enhanced if there were more parameters involved and if a different reference point were used. Technical problems do not contribute significantly to the weakness of the procedure as a screening test. The

Table I. Results of fetal BPD in 43 SGA newborn infants

Measurement	No. of SGA cases	Predicted	
		No.	%
1. Single BPD	24	13	54
2. BPD	19	10	53
Total	43	23	53

BPD is probably the most accurate measurement that can be obtained antenatally. Rather, this discrepancy in all likelihood is a reflection of the actual clinical pathology.

It is well established now that low birth weight by itself is not an accurate indicator of gestational age. The small-for-gestational-age neonates and those that are small, but appropriate for gestational age, must be distinguished by other clinical manifestations. Some SGA babies are proportionately small in weight and length and show little in the way of malnutrition. They are best distinguished from the small for appropriate age or true premature by their more advanced neurologic development. The other type of SGA baby has a long and thin appearance because body length is not as diminished as the fat and over-all muscle mass. The head appears disproportionately large. In other words, the fetal head and skeletal measurements are late in being affected in the growth-retarded gestations of this type. Campbell[4] described these as the late-flattening type and they comprise two thirds of his series of SGA babies.

While the results in predicting SGA infants by ultrasonic measurements of the fetal BPD are disappointing, the authors have, nevertheless, clearly defined the limitations of this approach. However, ultrasonography is still *potentially* a very useful clinical adjunct for early diagnosis of intrauterine growth retardation. A number of laboratories are examining fetal chest and fetal trunk measurements and growth rates for their applicability to the problem. Moreover, the sophistication of ultrasound technology has recently reached a level where computer-assisted measurement of the volume of fetal organs is possible by using real-time, gray-scale, B scan ultrasound transducer arrays. Measurement of kidney volume and limb lengths are feasible now. Hopefully, the state of the art will continue to progress to the point where it will soon be possible to identify the growth-retarded fetus with predictable accuracy through serial measurements of those organs that are distinctly affected early in intrauterine dysfunction such as the liver, spleen, thymus, and adrenals. A change in the ratio of brain volume (an organ least affected) to liver volume (an organ most affected) for example, would constitute strong evidence for fetal malnutrition. Other exciting potentials for this approach to fetal medicine are obvious.

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DR. J. BAY JACOBS, Arlington, Virginia. My comments should not be considered critical of this presentation. However, I am concerned about two items, the first of which relates to the early interruption of pregnancy. It has always been my observation that a small or premature fetus stands a more favorable chance of survival, following spontaneous onset of labor. I feel that the mother and fetus are subjected to greater risk when pregnancy is artificially interrupted.

The second item of concern and interest to me relates to the size of the BPD as an index of fetal maturity in utero. During the last 40 or more years, I had been directing attention to the fact that the size of the BPD, measured with precision, is the most reliable index of fetal maturity. The average normal BPD measures 9.25 cm. There are variations, since this diameter may measure slightly less or perhaps more in different individuals. I have found that the size of the father's head usually has some influence, since an unusually large head in the father usually results in a larger fetal skull.

In the July, 1936, issue of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY, I was first to describe a simple, accurate, practical technique of lateral pelvic roentgenography in the standing posture, which is far superior to any previous technique, and has been pretty commonly followed because of the numerous advantages over lateral roentgenography in the recumbent posture, as had been the customary procedure.

Some of the authors had credited this technique to Thoms, since he began to utilize it after reading my publication. Thoms, a pioneer in x-ray pelvimetry, wrote to me in 1936, stating that he was favorably impressed and intended to follow my technique. He published many studies, but usually did not include my name in the bibliographies.

The lantern slides which I am about to show demonstrate the simplicity of the technique. You will note certain important characteristics revealed in the lateral view, such as the BPD, the true conjugate, the inclination of the pelvis, the pelvic architecture, size of

the sacrosciatic notch, depth of the pelvis, direction of the birth canal, the posterior sagittal diameter, etc.

It had been my custom to take these pictures near term, or even in labor, if there was indication. The films are easy to interpret. In the standing posture, the head has a tendency to engage with the BPD in the true conjugate. This occurs in about two thirds of all cases, regardless of the type pelvis. I do not mean within 1 cm. but within 1 or 2 mm. An inaccuracy of 1 cm. in the last trimester would be an error of 3 weeks' gestation.

Over the years, this was routine procedure in my Health Department Clinics. I had interpreted over 3,600 such films, which were borderline cases, and they numbered one twelfth of the clinic population.

Then I reviewed 761 random cases in which I noted the date the x-ray picture was taken, the size of the BPD on that date, and the date of spontaneous labor and delivery. Some experts in the U. S. Public Health Service then plotted a graph which was published in the Gray Journal and in Bishop's *Textbook of Obstetric Roentengenology*. Reference to the chart reveals the approximate number of days when one might expect delivery to occur, depending upon accurate and simple determination of the BPD.

DR. ERNEST W. PAGE, New Brunswick, New Jersey. Dr. Queenan feels that he can make the diagnosis of intrauterine growth retardation with reasonable accuracy. Having done so, he did not discuss possible therapy other than preterm delivery.

At a Ross Conference, in 1969, I suggested that if a diagnosis of uteroplacental insufficiency could be made with accuracy, it should be possible to infuse appropriate nutrients into the amniotic fluid. The fetus would therefore imbibe most of his nutritional needs along with his usual daily intake of some 400 ml. of amniotic fluid.

We could not secure approval to try this on human subjects, although subsequently a Belgian investigator did so with apparently good results. Dr. Glendening and I did, however, feed rabbit fetuses in utero with an indwelling stomach tube. The formula contained yeast protein labeled with carbon-14, and we noticed that the proteins of the fetal brain and liver contained 20 to 100 times as much of the label as those of the littermates. Intrauterine fetal feedings, therefore, may become a useful mode of therapy.

Another form of treatment is enforced rest in the lateral recumbent position, togehter with appropriate therapy of any existing hypertension. This should afford maximal uteroplacental blood flow with improved intrauterine nutrition, and possibly avoid the necessity for preterm delivery with its attendant hazards.

DR. QUEENAN (Closing). I would like to thank the discussants for their provocative questions. Dr. Thiede, in our determination of the normal BPD's we did do an examination of the neonate, including an assessment of maturity. If the neonate was dysmature, we would eliminate the data. But if we were at odds with the gestational date by 1, 2, or 3 weeks, we still included the data in our series because this was a prospective study. We felt very strongly that if we had a last menstrual period that was accurate on the first examination, and the uterine size was consistent with it within plus or minus 1 week, that we were on solid grounds. Any prospective study of this sort has problems. As a matter of fact, Campbell's study eliminated all patients unless they delivered within the expected date of confinement plus or minus 1 week, which, of course, would eliminate all the normal patients that have delivered at 37, 38, 41½, or 42 weeks, which introduces some bias into a study.

I believe that we probably could have improved things by changing our percentile by using Usher's or other standards obtained at sea level. I think the question becomes a philosophical question of how wide do you want the gates open, and how many patients are you going to consider suspect for intrauterine growth retardation. I believe that there are great limitations to using this method alone for detecting all intrauterine retardation. There is a need for additional or adjunct diagnostic modalities. In Mann's study, he relied most heavily on urinary estriols. Sir Lance Townsend presented some data showing that plasma estriols are extremely helpful in detecting the SGA baby. In addition, fundal height and other factors may be helpful.

I believe that, with the advent of the new generations of ultrasound instruments, we are going to be able to evaluate differential growth much better—for instance, by the size of the chest vs. the size of the head. This is being done presently but it will be done much more accurately in the future.

Dr. Jacobs, I consider the introduction of x-ray BPD was an excellent advance in medicine. Unfortunately, in this setting we require serial determinations. Some of these patients had as many as five to seven ultrasound determinations, and I believe in trying to cone in on the very small number of patients who do have IUGR we need a method that is not invasive and nonradiation that may be repeated numerous times and be safe.

Infiltrative carcinoma of the vulva: Results of surgical treatment

GEORGE W. MORLEY, M.D.

Ann Arbor, Michigan

During a 40 year period 374 patients with infiltrative carcinoma of the vulva were evaluated. Some form of radical surgery was the treatment chosen for 278 patients. The absolute 5 year survival rates for all clinical stages treated with some form of radical surgery was 66.8 per cent with a corrected rate of 73.9 per cent. The corrected 5 year survival rate for patients treated with radical vulvectomy and groin lymphadenectomy approximated 80 per cent and if the regional lymph nodes were negative, it approximated 93 per cent. The size of the primary lesion and the incidence of regional lymph node metastases seemed directly related. Lymph node involvement itself significantly reduced the chance for survival. Furthermore, the inaccuracy of preoperative evaluation of the groin lymph nodes approximated 25 per cent. The assumption that a lesion is "early" does not justify less than radical surgery. Conversely, the "advanced" lesion when geographically localized can be treated quite satisfactorily with pelvic exenteration. The treatment of choice is discussed.

EVEN THOUGH women with cancer of the vulva are usually past middle age and often have associated medical problems, they show remarkable tolerance for the radical vulvectomy and regional lymphadenectomy required for effective treatment of the malignant lesion. This surgical method came into wide use after the studies of Taussig[1] and Way[2] demonstrated its superiority, and it has now been tested for well over a quarter of a century.[3–6] The result is that since 1940 a 5-year survival rate of about 10 per cent[7] has increased to about 90 per cent, among patients with vulvar carcinoma promptly and adequately treated by radical surgery.

When the lesion is detected reasonably early, appropriate treatment is applied without delay, and rehabilitation problems are handled to the patient's benefit, the patient's chance for a disease-free existence in the future is often more favorable than for a number of other malignant neoplasms seen by the practicing physician. Unfortunately, patient delay and physician mismanagement, despite concerted educational efforts, still act to decrease the chance of survival for many of these patients.[8, 9]

This report covers 374 cases of infiltrative carcinoma of the vulva recorded at the University of Michigan Medical Center in the 40 years from January, 1935, to July, 1975. All charts were reviewed by the author, and the T-N-M* and clinical staging system as most recently published by FIGO[10] was applied to all cases studied.[11] From this series, the major issues arising for discussion include the over-all survival rates as related to the various radical surgical procedures used, the significance of regional lymph node metastases, the error in preoperative evaluation of groin lymph-node–bearing regions in relation to the postoperative findings, the interpretation of the "early" and "advanced" lesions of the vulva and the benefits and limitations of bilateral pelvic lymph-node dissection.

Clinical evaluation and histopathologic features

Although a total of 404 patients with infiltrative vulvar malignancies were seen on the Gynecology Service at the University of Michigan Medical Center during the specified period, 30 of these were excluded

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*T-N-M designates the pretreatment evaluation of the primary Tumor, regional lymph Nodes, and remote Metastases: FIGO is the International Federation of Gynecology and Obstetrics.

from our final analysis because their invasive lesions were other than carcinoma. Of the 374 patients with infiltrative carcinoma, 278 were treated with some form of radical pelvic surgery and are evaluated in this report (Table I).

The ages of the patients ranged from 20 to 94; the median age was 63. Age itself is not considered a basic deterrent to the operation, nor do we rule out formerly designated "high-risk" patients with medical conditions such as a combination of hypertension, obesity, and diabetes mellitus. On the other hand, these medical illnesses must of course be taken into account in the over-all management of a given case—diabetes mellitus, for example, poses a threat because of its frequency of occurrence and its inherent effect on wound healing. Of the patients in our series, 14.4 per cent were under treatment for diabetes mellitus.

When the patients were seen in our hospital, the initial complaint in most cases (74.8 per cent) was the presence of a mass or growth in the vulvar area. Pruritus vulvae (38.8 per cent), localized pain (19.8 per cent), perineal bleeding (12 per cent), and a mass in the groin (1.9 per cent) were also complaints reported.

With respect to associated vulvar disease, it was estimated that almost half the malignant lesions were related to some type of previously existing chronic vulvar dystrophy (Table II). It was also disconcerting to note that even though carcinoma of the vulva is a surface lesion which is readily accessible to early diagnosis and treatment, still more than 30 per cent of the patients had conditions such as a sore, discharge, or itching for more than a year before seeking medical attention. Hopefully, patients now entering the "older age group" will not be hampered by the same embarrassment, sensitivity, and modesty in seeking medical advice that marked previous generations of women.

Even more disconcerting was the fact that the first physician to see the patient often was responsible for delay in diagnosis and treatment. About a third of the patients were subjected to topical therapy for 3 to 6 months before a biopsy of the lesion was performed. Moreover, a significant number of patients had been treated inappropriately, with a modified surgical approach, before they were seen in our hospital. The concept that small and therefore more favorable lesions can be treated with lesser surgical therapy was probably responsible for preventable deaths in many instances.

The different histologic types of infiltrative carcinoma of the vulva were also studied. Approximately 85 per cent of the lesions were histologically graded as well-differentiated, cornifying, infiltrative, squamous-cell carcinoma of the vulva. Microinvasive disease, as such, was not considered as a pathologic entity in this study. A more detailed analysis of this material has been reported elsewhere.[12]

Table I. Four types of radical surgery used in treatment of infiltrative carcinoma of the vulva

Type of surgery	*1935-1970*		*1935-1975*	
	No.	*%*	*No.*	*%*
Radical vulvectomy only	37	16.2	42	15.1
Radical vulvectomy plus groin lymphadenectomy	157	68.6	193	69.4
Radical vulvectomy plus groin and pelvic lymphadenectomy	23	10.0	29	10.4
Pelvic exenteration	12	5.2	14	5.1
Total	229	100	278	100

Table II. Incidence of associated vulvar diseases in 374 patients with infiltrative carcinoma of vulva

Associated vulvar disease	*No. of patients*	*Per cent of patients*
"Leukoplakia,"	137	36.6
"Kraurosis"	31	8.3
Condyloma acuminatum	18	4.8
Lymphopatheum venereum	4	1.1
Lymphogranuloma inguinale	2	0.5
Total	192	51.3

The treatment method

Preoperative care. After an initial complete medical evaluation, the patients are presented to the Gynecology Tumor Conference for final diagnosis, preoperative staging, and recommended disposition. Almost all patients are given prophylactic heparin therapy, and some are given antibiotics prophylactically if this seems indicated.

Operative technique. In performing this operation one must adhere to the basic principles of cancer surgery as originally set forth by Basset[13] in 1912 and later modified by others[1, 14–16] by carrying out en bloc dissection and insuring adequate borders around the neoplastic mass. Whereas the technical details are the subject of another paper, some of the surgical principles should be presented at this time. One must be familiar with the surgical anatomy of the various areas so that "accidents of surgery" can be avoided. On the other hand, one must be careful not to confuse the surgical approach with that of an anatomic dissection. Anatomists describe the planes, layers, and locations in differentiating the various lymph-node groups; surgeons must simply keep in mind two designated areas

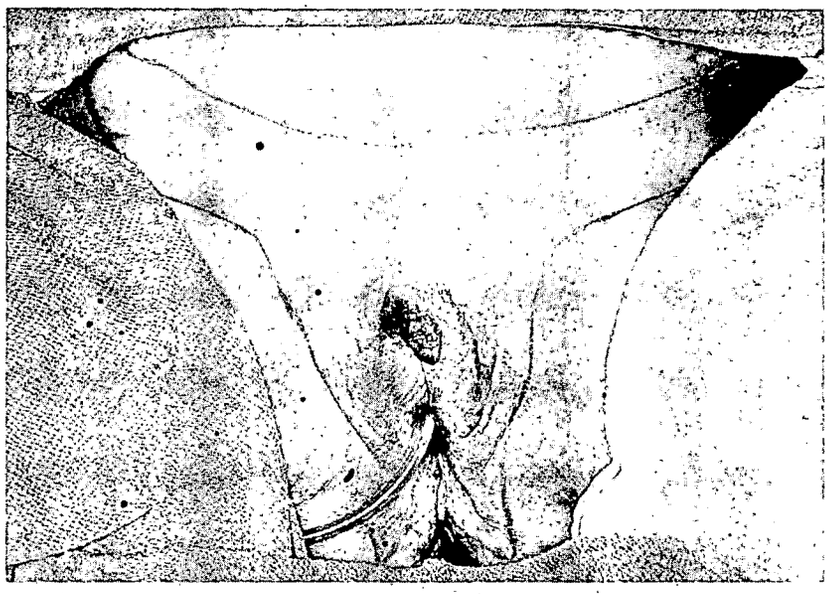


Fig. 1. Trapezoid or butterfly incision.

as they relate to the surgical treatment of infiltrative carcinoma of the vulva, and consider bilateral groin and pelvic lymphadenectomy as two distinct areas of dissection. In most cases the entire operation can be performed as a one-stage procedure.

At operation, the patient is placed in the so-called "ski position"[17] so that the lower abdominal and perineal regions can be dissected without repositioning the patient. A trapezoid or butterfly-like incision is utilized (Fig. 1) in performing the en bloc dissection. A full-thickness block of tissue is dissected off the underlying fascial areas and one must remember that the rectus abdominis fascia of the abdominal wall, the fascia lata of the thigh, and the inferior fascia of the urogenital diaphragm are all in the same fascial plane and are in contact with each other at the symphysis pubis and rami. This full-thickness dissection is designed to maintain circulation in the remaining flaps. We no longer undermine the skin flaps, because in our experience this led to unsatisfactory wound healing.

The groin dissection is simplified by elevating the fibroadipose tissue off the underlying fascia lata in the following manner. A medial incision at the top of the labiocrural line is carried down onto the fascia overlying the adductor longus muscle. By blunt dissection the fibroadipose tissue is separated off the fascia lata from medially to laterally and anterior to the cribriform fascia overlying the femoral triangle. This anterior block of tissue contains the superficial inguinal lymph-node–bearing tissue and the greater saphenous vein. The elevation of this tissue allows for easier dissection of the area, since the femoral vessels are posterior to the dissecting finger. The femoral sheath is then incised longitudinally in opening the femoral triangle. The fascia lata is removed only if the nodes or the cancer are adherent to it. The femoral triangle is cleared of the deep inguinal (femoral) lymph nodes, including Cloquet's node, which anatomically speaking is considered the sentinel node. It is not necessary to clean the femoral nerve and its branches since it lies outside the femoral sheath.

The great vessels of the femoral triangle must be protected either by closure of the femoral sheath at the site of the previous incision or by transplantation of the sartorius muscle medially with fixation to the inguinal ligament. When possible, the bilateral groin lymph-node dissections are carried out simultaneously by "double-teaming" the procedure as an operative time-saving step.

The necessity for bilateral groin lymph-node dissection is well documented.[18, 19] The area is rich with lymphatic vessels, and liberal anastomosis across the midline allows for contralateral drainage of the vulvar tissues and resultant metastasis in some cases. The groin dissection is carried out bilaterally before any consideration is given to pelvic lymphadenectomy.

At this point a decision must be made concerning pelvic lymphadenectomy. On occasion, an exploration of the pelvic lymph nodes may be all that is indicated. But if pelvic lymphadenectomy is planned, a parallel incision of the aponeurosis of the external oblique muscles is made along the inguinal ligament. The peritoneum is then reflected superiorly and medially to give adequate retroperitoneal exposure for dissec-

tion of the iliac, hypogastric, obturator, and common iliac lymph nodes. Seldom is there an indication for drainage of the retroperitoneal space.

The en bloc resection is continued down onto the inferior fascia of the urogenital diaphragm and into the ischiorectal fossa to include the vulvar and perineal tissues; whether cautery or sharp dissection is used depends on the surgeon's preference. This completes the dissection and once hemostasis is obtained, the wound is ready for closure. The portions of the urethra and vaginal wall are included in the en bloc dissection when indicated.

The wound is closed in a variety of ways. In over 90 per cent of our cases the perineal, vulvar, and groin wounds are closed primarily; however, there is an area of about 7 by 8 cm. located suprapubically that cannot be covered without unusual tension on the suture material. This area is allowed to heal secondarily by granulation, rather than by skin grafting. Usually no drains are inserted in the wounds, but a pressure dressing is applied so as to eliminate possible "dead spaces" with the tendency to retain secretions. This compression dressing is left in place for 18 to 48 hours.

Postoperative care. Most important for the patient is the expert care which is provided by our interested and dedicated nursing staff. After the operation, the patients are kept in a semi-Fowler's position and allowed only "log rolling" for 48 hours. After the initial dressing change at about 48 hours, the wound is left exposed and the patient is gradually ambulated. Sitz baths and whirlpool physiotherapy are started shortly thereafter and urinary catheter drainage can be discontinued in 4 or 5 days. Primarily, we depend on pressure dressings; however, if Hemovac or Penrose drains have been used, they are removed within 72 hours.

Complications The incidence of major complications is not particularly significant. The complication encountered most frequently was wound breakdown which occurred to some degree in over 50 per cent of the cases.

Occasionally a major bleeding episode is encountered during the postoperative period; however, the great vessels are not involved, because, if necessary, the sartorius muscle has been transplanted as a roof over this otherwise unprotected area. In our series, six patients experienced blood loss of hemorrhagic proportions postoperatively which was thought to be related either to infection involving lesser vessels or to an untoward response to prophylactic anticoagulant therapy.

Lymphedema of the lower extremities is encountered quite frequently but is usually not of major

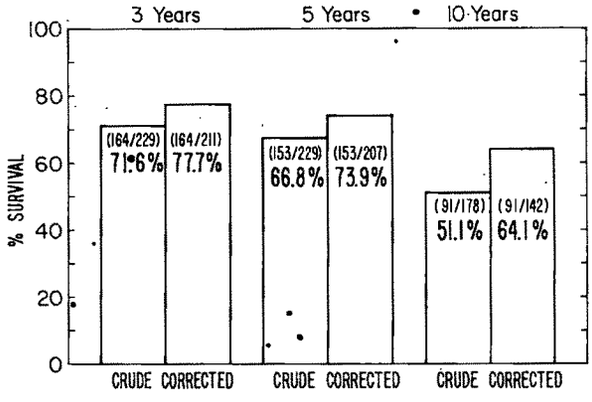


Fig. 2. Survival rates (3, 5, and 10 years) for all stages treated with radical pelvic surgery.

consequence; it can best be corrected by the use of elastic stockings for 3 to 6 months. It is thought that even though lymph nodes can never regenerate, the lymphatic channels themselves can regenerate very quickly. Nevertheless, if conservative therapeutic measures are not carried out the lymphedema may become chronic and perhaps permanent.

The development of a lymphocyst in the groin area is an infrequent occurrence, but we suspect it occurred in our series more often than was recorded on the charts. It probably can be prevented by careful ligation of all the lymph-bearing tissue during the groin dissection. It is treated by the application of pressure dressings to the area, with intermittent aseptic aspiration of the fluid. Seromas and hematomas which occasionally develop are treated in a similar manner.

Of considerable importance among these patients is the occurrence of late complications—difficulties that arise some weeks or months after the operation. Sometimes, patients complain of perineal numbness or stiffness, lack of control over urinary direction at micturition, and pulling of the scar around the vaginal opening. Occasionally a patient complains of dyspareunia, because of significant vaginal stenosis.

Symptoms related to stress incontinence and the development of a cystocele or rectocele are sometimes reported by these patients. These conditions reflect the loss of the supports of the lower end of the vagina, and consequent enlargement of the introitus. One must remember that these findings may simply reflect the increased frequency of pelvic visceral prolapse among older women.

In five cases in our series the urethral orifice became obliterated by the development of severe cicatricial changes around the vaginal opening. The resultant urinary blockage was significant in two cases, and was easily corrected by vaginal dilation and external

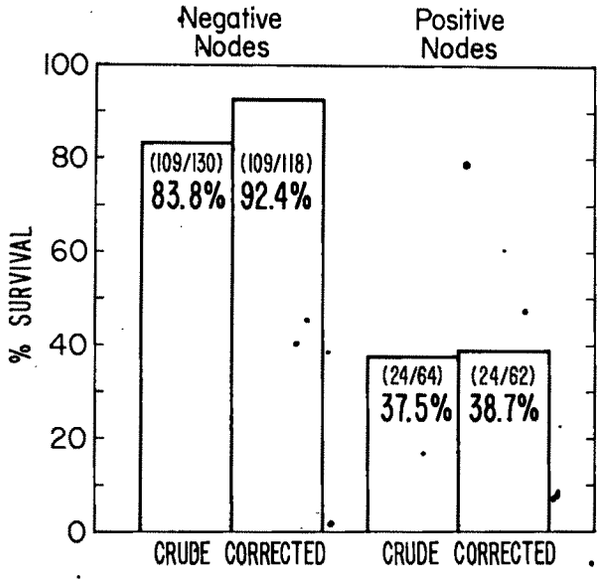


Fig. 3. Groin lymph node status: 5 year survival rate.

Table III. Crude and corrected 5 year survival rates for Stages I, II, and III when related to all forms of radical surgery

	Crude 5 yr. survival rates		*Corrected 5 yr. survival rates*	
Stage	*No.*	*%*	*No.*	*%*
I	66/85	77.6	66/77	85.7
II	54/62	87.1	54/57	94.7
III	32/81	39.5	32/72	44.4

urethral meatotomy. One patient developed a recto-vaginal fistula two months after the operation.

Results of analysis

All statistical analyses were carried out in the University of Michigan Computer Center. It is important to note that the results are given in both "crude" (uncorrected) and "corrected" form. Because of the age of these patients and the associated death rates in the general population for this age group, special attention was directed toward correcting our data for "intercurrent deaths"—that is, deaths among these patients that were not related to the carcinoma for which they had received surgical treatment.

Survival rates. It was determined that 229 of the 278 patients were treated before January, 1970, and thus could be analyzed for 3, 5, and 10 year survivals (Fig. 2). Considering the over-all survival, including all stages of disease and all four forms of radical surgery, 153 of the 229 patients or 66.8 per cent were living at 5 years. When corrected for intercurrent deaths, the total number was 153 of 207 patients, with a 5 year survival rate of 73.9 per cent. In considering those patients at 5 years who were disease-free, 137 of 212, 64.2 per cent were alive and well; the corrected rate was 139 of 190 patients or 71.6 per cent. The over-all 3 and 10 year survival rates are similarly recorded.

The crude 5 year survival rate for patients having negative lymph nodes (Fig. 3) (either groin or pelvic) was 109 of 130 patients or 83.8 per cent, with a corrected rate of 92.4 per cent. For patients having positive lymph nodes, the crude 5 year survival rate was 24 of 64 or 37.5 per cent; the corrected value was 38.7 per cent. These figures are statistically significant at the 1 per cent confidence level by Fisher's Exact Binomial Test.

Evaluation of the size of the primary lesion initially suggests that there is no significant association with decade of detection and institution of therapy; however, this material is biased, since only those patients

Table IV. Crude and corrected 5 year survival rates for all stages combined when related to specific form of radical surgery

	Crude 5 yr. survival rates		*Corrected 5 yr. survival rates*	
Type of surgery	*No.*	*%*	*No.*	*%*
Radical vulvectomy only	21/37	56.8	21/29	72.4
Radical vulvectomy plus groin lymphadenectomy	115/157	73.2	115/146	78.8
Radical vulvectomy plus groin and pelvic lymphadenectomy	9/23	39.1	9/20	45.0

Table V. Crude and corrected 5 year survival rates for Stage I disease treated by radical vulvectomy only vs. radical vulvectomy plus groin lymphadenectomy

	Crude 5 yr. survival rates		*Corrected 5 yr. survival rates*	
Stage I treatment	*No.*	*%*	*No.*	*%*
Radical vulvectomy only	13/22*	59.1	13/16†	81.3
Radical vulvectomy plus groin lymphadenectomy	53/62	85.5	53/60	88.3
Total no. of patients	84		76	

*Difference at significance level of p = 0.0133.
†Difference not significant at p = 0.3516.

Table VI. Crude and corrected 5 year survival rates for Stage II disease treated by radical vulvectomy plus groin lymphadenectomy vs. radical vulvectomy plus groin and pelvic lymphadenectomy

	Crude 5 yr. survival rates		*Corrected 5 yr. survival rates*	
Stage II treatment	*No.*	*%*	*No.*	*%*
Radical vulvectomy plus groin lymphadenectomy	43/47*	91.5	43/44*	97.7
Radical vulvectomy plus groin and pelvic lymphadenectomy	3/5	60.0	3/4	75
Total no. of patients	52		48	

*Neither crude nor corrected 5 year survival rates were significantly different: p = 0.0958 and p = 0.1613, respectively.

Table VII. Crude and corrected 5 year survival rates for Stage III disease treated by radical vulvectomy plus groin lymphadenectomy vs. radical vulvectomy plus groin and pelvic lymphadenectomy

	Crude 5 yr. survival rates		*Corrected 5 yr. survival rates*	
Stage III treatment	*No.*	*%*	*No.*	*%*
Radical vulvectomy plus groin lymphadenectomy	19/48*	39.6	19/42*	45.2
Radical vulvectomy plus groin and pelvic lymphadenectomy	6/17	35.3	6/15	40.0
Total no. of patients	65		57	

*Neither crude nor corrected 5 year survival rates were significantly different: p = 0.4955 and p = 0.4834, respectively.

treated with some form of radical surgery were statistically analyzed in this way. It is of some interest, however, to note that more patients were treated with palliative vulvectomy or had lesions too extensive for surgical therapy in the 1940's than in the 1960's, implying that lesions seen today are somewhat smaller than those seen 20 to 30 years ago. The mean diameter of T_2 lesions treated surgically in the decade 1940-49 was 5.5 cm., and the mean for 1960-69 was 4.2 cm.

Clinical stages and radical surgical procedures. When cases of clinical Stages I, II, and III lesions were analyzed for 5 year survival rates in relation to *all* forms of radical surgery combined (Table III), 66 of 85 patients in Stage I, or 77.6 per cent (uncorrected), and 85.7 per cent (corrected) survived 5 years. In Stage II, 54 of 62 or 87.1 per cent (uncorrected) and 94.7 per cent (corrected) survived 5 years. In Stage III, the respective figures were 39.5 per cent, and 44.4 per cent surviving 5 years.

When the specific surgical operations were analyzed in relation to all clinical stages combined (Table IV), the crude 5 year survival rate was 56.8 per cent and the corrected rate was 72.4 per cent for the 37 patients treated with radical vulvectomy alone. Of the 157 patients treated with radical vulvectomy and groin lymph node dissection, 115 or 73.2 per cent survived 5 years; the corrected ratio was 78.8 per cent. For the 23 patients treated with radical vulvectomy plus groin lymph node and pelvic lymph node dissection, the 5 year survival was 39.1 per cent uncorrected and 45.0 per cent corrected.

Of 84 patients in Stage I, 22 were treated with radical vulvectomy alone, and 62 by radical vulvectomy and groin lymph node dissection (Table V). The crude 5 year survival rates were 59.1 and 85.5 per cent, respectively. This is significantly different at the 2 per cent level by Fisher's test (P = 0.0133). However, the corrected rates were 81.3 and 88.3 per cent, respectively, and this difference between operations was not significant. The higher intercurrent death rate among patients undergoing only vulvectomy suggests that many of these patients were "at risk" for various reasons.

Of 52 patients in Stage II, 47 were treated with radical vulvectomy and groin lymph node dissection, and five with radical vulvectomy plus both groin and pelvic lymph node dissection (Table VI). The uncorrected 5 year survival rates were 91.5 and 60.0 per cent, respectively. The corrected rates were 97.7 and 75 per cent, respectively. Neither the crude nor the corrected rates differed significantly for the two types of operation, but the numbers are small in the latter group. *We must remember without getting lost in the statistics, that when Stage I and Stage II disease were treated with radical vulvectomy and groin lymph node dissection that the corrected 5 year survival rate approximately 90 to 95 per cent.*

Of 65 patients in Stage III, 48 were treated by radical vulvectomy and groin lymph node dissection and 17 by radical vulvectomy and both groin and pelvic lymph node dissection (Table VII). The uncorrected 5 year survival rates were 39.6 and 35.3 per cent, respectively; the corrected rates were 45.2 and 40.0 per cent. The survival rates for the two types of operation did not differ significantly, which would suggest that there is very little promise for improved chance of survival when pelvic lymph node dissection is added to the procedure. Four of the 11 postoperative deaths

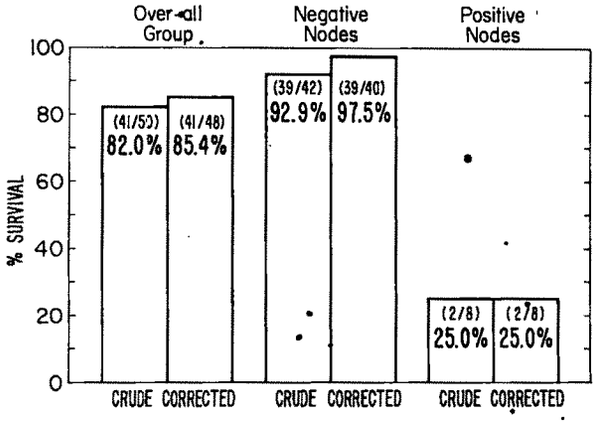


Fig. 4. T_1 N_0 M_0—groin lymph node status: 5 year survival rate.

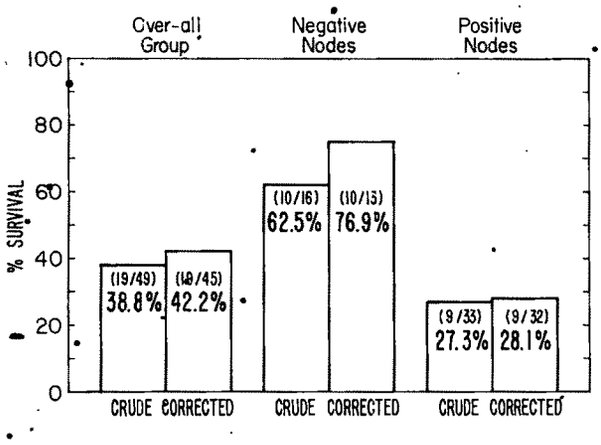


Fig. 5. T_2 N_2 M_0 lesions—groin lymph node status: 5 year survival rate.

occurred in the group undergoing pelvic lymphadenectomy.

Lymph node metastasis. Although not always directly related, the size of the primary lesion and the incidence of regional lymph node involvement seems important. T_1 lesions showed a 20.7 per cent incidence of lymph node involvement; for T_2 lesions the incidence more than doubled—44.8 per cent. The over-all incidence of positive groin lymph nodes was 37 per cent.

In addition, the presence or absence of lymph node metastases seems to have an important impact on 5 year survival (Fig. 4). Of the 50 patients classified preoperatively as T_1 N_0 M_0 ("early lesions"), the corrected 5 year survival rate was 85.4 per cent. Regional lymph nodes were negative in 42 of these patients, so that the corrected survival rate was 97.5 per cent. For the eight patients with positive nodes, the survival rate was 25.0 per cent. This difference was

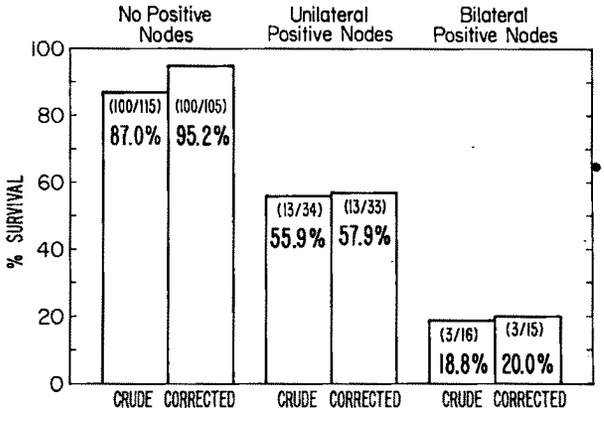


Fig. 6. T_1 and T_2 lesions—groin lymph node status: 5 year survival rate.

statistically significant at the 0.5 per cent confidence level.

Classified preoperatively as T_2 N_2 M_0 ("late lesions") were 49 patients (Fig. 5). The corrected 5 year survival rate was 42.2 per cent. For the 13 patients with negative groin lymph nodes the rate was 76.9 per cent and for the 32 patients with positive nodes the rate was 28.1 per cent. The difference associated with lymph node metastases was significant at the 2.5 per cent confidence level.

Looking at it from a different point of view, the combined T_1 T_2 lesions were evaluated in regard to no groin lymph node involvement, unilateral groin involvement, and bilateral groin involvement. Of 165 patients with a T_1 or T_2 lesion, 115 had no groin lymph node involvement, 34 had unilateral involvement, and 16 had bilateral involvement (Fig. 6). Of those patients with no groin lymph node involvement 100 or 87.0 per cent survived 5 years; the corrected rate was 95.2 per cent. Of the 34 patients with unilateral nodal involvement, 55.9 per cent survived 5 years; the corrected rate was 57.6 per cent. Twenty-one of these patients had just one positive groin lymph node and in this specific group there was an 81 per cent uncorrected 5 year survival rate. Of the 16 patients with bilateral nodal involvement, the corrected 5 year survival was 20.0 per cent. Twelve of these patients had only a unilateral primary lesion.

A relative small number of patients (excluding those undergoing pelvic exenteration) were treated by pelvic lymphadenectomy in addition to radical vulvectomy and groin lymph node dissection. Of these 29 patients, 23 were treated before January 1970, and only six in this group or 26 per cent had positive pelvic lymph nodes. All six of the patients who had pelvic lymph node involvement also had groin lymph node involve-

Table VIII. Errors in "T-N-M system" when related to pathology review of regional lymph nodes: 234 patients analyzed

T-N-M classification (234 patients)	*Negative nodes*	*Positive nodes*	*Per cent error*
Total—120 patients:			
$T_1 N_0 M_0$	50	11	18.0
$T_2 N_0 M_0$	38	10	20.8
$T_3 N_0 M_0$	9	2	18.2
Over-all error		23/120	19.2
Total—34 patients:			
$T_1 N_1 M_0$	10	4	28.6
$T_2 N_1 M_0$	13	3	18.8
$T_3 N_1 M_0$	4	0	0.0
Over-all error		7/34	20.6
Total—80 patients:			
$T_1 N_2 M_0$	5	2	71.4
$T_2 N_2 M_0$	18	43	29.5
$T_3 N_2 M_0$	4	8	33.3
Over-all error		27/80	33.8
Over-all error and total		57/234	24.4

Table IX. Errors in clinical staging system when related to pathology review of regional lymph nodes: 205 patients were analyzed

Clinical staging	*Negative nodes*	*Positive nodes*	*% Error*
Stage I	58	15	20.5
Stage II	51	13	20.3
Stage III (excluding T_3 lesions)	22	46	32.4
Total		205	
Over-all error		50/205	24.4

ment. The uncorrected 5 year survival rate for these six patients was 1 of 6, or 16.7 per cent. The corrected rate was 25 per cent. For the 17 patients with no lymph node involvement the crude 5 year survival rate was 47.1 per cent and the corrected rate was 57.1 per cent.

The inaccuracy of preoperative interpretation of nodal involvement has been recognized for some time. The "N" portion of the "T-N-M" system (N_0—no groin lymphadenopathy; N_1—no suspicious groin adenopathy; N_2—suspicious groin adenopathy) was analyzed for accuracy in all cases where nodal tissue was available for pathology review (Table VIII). Of the 234 cases analyzed, the over-all *error* for groin lymph node involvment in preoperative T-N-M classification of patients undergoing radical surgery with regional lymph node dissection was 24.4 per cent.

The preoperative clinical staging system outlined in the FIGO classification also was analyzed for accuracy in 205 cases (Table IX). The over-all *error* for groin lymph node involvement in preoperative clinical staging was 24.4 per cent.

Table X. Analysis of 14 patients treated with some type of pelvic exenteration

Type of surgery	*No.*	*Per cent*
Total pelvic exenteration	3	21.4
Posterior pelvic exenteration	9	64.3
Anterior pelvic exenteration	2	14.3
Over-all 5 year survival rates	8/12	66.6

Table XI. Postoperative mortality rate in the series of 278 patients

Type of death	*No.*	*Per cent*
Surgical	5	1.8
Radical vulvectomy only	3	
Radical vulvectomy plus groin and pelvic lymphadenectomy	2	
Hospital	6	2.1
Radical vulvectomy only	1	
Radical vulvectomy plus groin lymphadenectomy	3	
Radical vulvectomy plus groin and pelvic lymphadenectomy	2	
Cause of death	11	
Septicemia	5	
Pulmonary embolization	3	
Cerebral vascular accident	3	

"Early" and "advanced" lesions. Since some controversy exists in regard to the appropriate treatment of the so-called "early" lesion, patients classified as $T_1 N_0 M_0$ were specifically analyzed. Over the 40 year period 61 patients in this category were treated with radical vulvectomy and groin lymph node dissection even though regional lymph node involvement was thought not to exist at the time of preoperative evaluation. Eleven or 18 per cent of these patients had positive groin lymph nodes either unilaterally or bilaterally.

Conversely, 14 patients had such "advanced" lesions that some type of pelvic exenteration was considered the appropriate treatment (Table X). Nine patients were treated with posterior pelvic exenteration; three were treated with total pelvic exenteration and two with anterior pelvic exenteration. Of the 12 patients operated upon 5 or more years ago, eight were alive and well at 5 years for a 5 year survival rate of 66.6 per cent. The other four patients died with disease within 5 years.

Recurrence. In evaluating the success or failure of the surgical procedures the recurrence rates must be evaluated. Of the 229 patients treated before 1970, 71 or 31 per cent had a recurrence of disease within the first 5 years after the operation. Moreover, from 1935 to 1950 the recurrence rate was 37.7 per cent and from 1960 to 1970 (when more radical en bloc dissection was instituted) the recurrence rate dropped to 26.4 per cent.

Vital statistics. The operating time for the three major radical procedures ranged from 1½ to 5 hours, with a median of 2 hours and 45 minutes. Estimated blood loss ranged from 300 to 3,500 ml., with a median of 1,125 ml. Postoperative hospital stay ranged from 9 to 58 days with a median of 21 days.

In the series of 278 patients there were 11 postoperative deaths. (Table XI). There were five deaths within the first 30 days; three patients died from pulmonary embolism, one from cerebrovascular accident, and one from septicemia, thus yielding a 1.8 per cent surgical mortality rate. Six patients died before discharge from the hospital; four of whom died from septicemia and two from cerebrovascular accident. Therefore, there was a 2.1 per cent hospital mortality rate. Since 1968, however, there have been no surgical or hospital deaths following radical operations on the vulva.

Comment

Compared with the total number of gynecology patients with infiltrative malignant tumors considered by the Gynecology Tumor Conference since January, 1935, the 404 patients with infiltrative vulvar malignancy represent an incidence of 4 per cent. Previously reported conclusions are confirmed through this report, and it is suggested that they form the basis for criteria for the future treatment of infiltrative carcinoma of the vulva. Analysis of this 40 year series discloses a well-defined interrelation between infiltrative carcinoma of the vulva and chronic vulvar dystrophy, since almost half of the patients had a history of pre-existing chronic vulvitis. Chronic vulvar dystrophies, therefore, should be considered precancerous lesions.

Patient delay and physician mismanagement still must be stressed in our educational efforts, if we are to reduce the time between onset of symptoms and institution of therapy. In the study reported here, about a third of the patients had had symptoms for over a year, and an equal number had been treated medically with various lotions, potions, and ointments for 3 to 6 months before biopsy study was carried out. If premalignant lesions were routinely managed aggressively and followed carefully, the incidence of malignant tumors of the vulva could probably be reduced by about half. Corollary to this is the possibility that reduced patient delay and earlier diagnosis of disease might significantly raise the survival rate for this disease.

While it is generally agreed that neither radiation therapy alone nor conservative surgical procedures can effectively control infiltrative carcinoma of the vulva, controversies still exist as to exactly what does constitute adequate therapy. Some authors [20, 21] advocate a relatively conservative and individualized approach to the so-called early lesion, the treatment being tailored to meet the needs of the patient. Age in itself, however, and the presence of medical complications usually associated with older patients have not been regarded as strong contraindications to radical surgery.

Many authors[5, 22] recommend radical vulvectomy and bilateral groin lymph node dissection as the fundamental form of therapy and usually as a one-stage procedure. Whether the pelvic lymph nodes should be removed is another matter—some authors[4, 15] suggest that bilateral pelvic lymph node dissection be carried out only when certain criteria are met, but others[6, 23] believe this dissection should be part and parcel of the surgical treatment, routinely.

Whatever the specific philosophy one teaches, the principle of radical surgery must be endorsed if we are to significantly improve the chances of survival. At the University of Michigan radical vulvectomy and bilateral groin lymph node dissection, with or without pelvic lymph node exploration, is the fundamental therapeutic approach to most infiltrative lesions of the vulva. Up to now, pelvic lymph node dissection has been considered only in patients with clitoral lesions, groin lymph node involvement, melanoma of the vulva, adenocarcinoma of the vulva, and vaginal or urethral extension of the vulvar disease. Of course, pelvic lymph node dissection was performed in all patients treated with pelvic exenteration.

The 278 patients with infiltrative carcinoma of the vulva were treated with one of four types of radical surgery to include: radical vulvectomy only, radical vulvectomy with groin lymph node dissection, radical vulvectomy plus groin lymph node and pelvic lymph node dissection, and some form of pelvic exenteration during the 40 year period from January 1, 1935, to July 1, 1975. Thus, 229 patients are the subject of 5 year review.

In our series of 229 patients treated with some form of radical surgery prior to January, 1970, the over-all 5 year survival rate (corrected for intercurrent deaths) was 73.9 per cent. For all stages of disease with negative regional nodes, the rate was 92.4 per cent; for cases

with positive regional nodes the rate was 28.7 per cent. This in itself suggests the paramount importance of the method of evaluating and treating regional lymph nodes. The over-all corrected 10 year survival rate was 54.1 per cent.

When radical vulvectomy and groin lymph node dissection was the treatment of choice for Stage I and Stage II disease, the corrected 5 year survival rate was approximately 95 per cent; for Stage III the rate was 45 per cent. Moreover, when Stage I patients were treated with radical vulvectomy alone the survival rate was about 60 per cent. Such figures leave little room for doubt that the patient's best chance lies with the radical procedure mentioned here and described in detail above.

Although the series in which *pelvic lymphadenectomy* was added to the procedure was small (only 23 patients), it should be pointed out that of the six patients with positive nodes only one lived 5 years or more and none of these patients had positive pelvic lymph nodes without also having groin lymph node involvement. Three died of the disease within 5 years, and the other two died in the postoperative period. This suggests that the pelvic lymph node dissection added significantly to the operating time and the risk to the patient, without contributing significantly to the chance of survival. Some authors[24, 25] have reported the successful use of postoperative radiotherapy to the pelvic lymphatic system without the obvious risk of surgical or hospital death.

One has three options, then, in regard to the presence of positive groin lymph nodes or other existing indications for *pelvic lymph node treatment:* (1) extraperitoneal exploration and/or dissection of the pelvic lymph nodes, as part of the one-stage procedures; (2) transperitoneal pelvic lymphadenectomy at a later date, if indicated; or (3) postoperative pelvic irradiation, at dosage levels approximating 5,000 or 5,500 rads, to the whole pelvis. It appears to be an either-or program: Either you do the pelvic lymph node dissection, or you recommend postoperative irradiation. We must remember that pelvic irradiation is still regarded as an accepted treatment for infiltrative carcinoma of the cervix, and since cervical and vulvar malignancies are usually of the squamous-cell variety, there is good reason to also consider irradiation to the pelvic lymph nodes in infiltrative carcinoma of the vulva.

It is proposed, therefore, that a radical vulvectomy and groin lymph node dissection be the initial therapeutic approach to carcinoma of the vulva. If the groin lymph nodes (either superficial or deep) are positive, or if other existing criteria dictate, the patient should then undergo either *pelvic* lymphadenectomy or *pelvic* irradiation, according to the surgeon's viewpoint. Those recommending pelvic lymphadenectomy endorse the philosophy that infiltrative carcinoma of the vulva is a surgically treated disease; those recommending pelvic irradiation should adopt the belief that the external disease is treated by radical vulvectomy and bilateral groin lymphadenectomy and the internal disease by pelvic irradiation.

Whereas there is probably a direct relationship between the size of the primary lesion and the chance survival, the status of the regional lymph nodes seems to be an even more important prognostic indicator. The accuracy of physical diagnosis, however, is limited in determining the true nature of enlarged groin nodes and their appraisal by palpation has been controversial and subject to error. The over-all error of lymph node assessment in the T-N-M system and in the preoperative clinical staging approximated 25 per cent. Clinical speculation is intriguing, and pathologic confirmation is rewarding; however, in this medically sophisticated era, it is time to restrict this evaluation as follows: The preoperative descriptive T-N-M system should be considered an observation only and the definitive clinical staging should be reserved until all the facts are in postoperatively. This is not without precedence; carcinoma of the ovary and sometimes carcinoma of the endometrium are staged *after* surgical treatment! Simply stated, should a patient with T_1 N_0 M_0 disease who actually has positive regional lymph nodes be classified T_1 N_0 M_0 rather than T_1 N_2 M_0? Conversely, should a patient classified T_1 N_2 M_0 and thus Stage III disease, who actually has negative groin lymph nodes, be classified Stage III rather than Stage I? As shown in our study, the presence or absence of lymph node metastases is directly related to patient survival when adequate surgical therapy has been instituted. It is therefore recommended that some type of postoperative staging be given serious consideration.

Recently much attention has been focused on the so-called "early" lesion of the vulva, with the suggestion that lesser surgery be performed for lesser disease. In our review of T_1 N_0 M_0 lesions we found an approximate 20 per cent error in the preoperative classification of the regional lymph node status as compared to postoperative pathology examination. One must, therefore, continue to caution against the injudicious use of lesser surgical procedures in these patients.

On the other hand the "advanced" lesions should not be considered hopeless.[25, 27] Because of the extent and geographic location of the primary lesion in 14 of the patients in this study, some type of pelvic exenterative surgery was considered appropriate in an attempt to

control the disease. Of the 12 patients operated on 5 or more years ago, eight or 66.6 per cent are living and well without evidence of disease.

Because of the relatively high incidence of recurrence, periodic follow-up examination is mandatory for these patients. There was an approximate 33 per cent recurrence rate within the first 5 years from initial therapy and approximately 85 per cent of these recurrences occurred within the first 3 years. Most local recurrences can be treated successfully with a wide local excision.

At present there appears to be little benefit from the use of chemotherapeutic agents; however, immunotherapy using the transfer factor has shown promise in one patient who is alive and well 24 months after an exploratory operation disclosed an extensive primary lesion with bilateral involvement of both groin and pelvic lymph nodes as well as the para-aortic lymph nodes. Her condition was judged inoperable at that time, and irradiation was considered of limited value; therefore, the immunotherapy was instituted.

Finally, the patient must be psychologically supported not only during the immediate postoperative period but throughout the long rehabilitation period. We inflict significant injury on these patients when we do radical vulvar surgery, and it is our obligation to rehabilitate them so that they may live more nearly normal lives in the future. Some have referred to this procedure as a disfiguring operation; others as a mutilating one. Some day in the future, hopefully, some type of plastic reconstruction or "vulvoplasty" can be performed satisfactorily in this area.

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Discussion

DR. ABRAHAM F. LASH, Chicago, Illinois. My personal experiences over the same period of time (that is, 40 years) at the Cook County Hospital of Chicago form the basis for my discussion.

The importance of the preoperative study of each patient cannot be overemphasized. The various medical conditions in the elderly patients, such as anemia, obesity, diabetes, hypertension, and myocardial infarct, properly controlled by medical consultants, may con-

vert an operative risk to an acceptable surgical candidate. Also, an adequate complete study is needed to rule out asymptomatic primary malignancies as in the breast, gastrointestinal area, or other sites in the generative tract. In our series, a 5 per cent incidence of multiple primary cancers were found. Boutselis[1] reported 15 per cent coexisting malignancies.

Age was no deterrent to adequate surgery since it is extraperitoneal and not shock-producing. Our age group varies from 23 to 102 years. The most common precursor or associated pathology in the younger age group were condyloma acuminatum, requiring multiple and repeated biopsies to achieve a definitive diagnosis of carcinoma.

The clinical judgment for individualized therapy for a specific patient is based on an extensive surgical experience. Since carcinoma of the vulva is uncommon in the usual gynecologic practice, it seems logical to have these patients treated in well-organized cancer centers having such expertise.

Dr. Morley properly emphasized the inaccuracy in determining lymph node involvement by palpation (25 per cent). Kelly[3] reported that 28.8 per cent of the patients seen with apparently normal lymph nodes were found to have lymph node metastases. Lymphangiograms have not proved to be as helpful as anticipated for demonstrating involved nodes. But intrasurgical films taken after lymphangiograms before wound closure audit the completeness of the lymphatic tissue and nodes dissection. The patient is positioned over the Bucky casette and after the surgery is completed a film is taken which permits localization of any overlooked residual nodes, which may then be removed. This technique is especially advisable for those gynecologists performing their first radical vulvectomies and groin dissections. The decrease in the surgical mortality rate and increase in the 5 year survivals and cures over the last 50 years has been due to the thoroughness of surgical removal of the cancer-bearing tissue (radical vulvectomy) and the related lymphatics and nodes—(from Taussig[6] (30 to 50 per cent) to Morley (66 to 90 per cent). The blood banks, antibiotics, and anticoagulants have contributed toward improving the 5 year cures as well as survivals.

To prevent constriction of the vaginal vestibule and/or prolapse of the anterior vaginal wall and bladder or posterior wall with resulting rectocele, mobilization of the lower third or half of the vagina has been found helpful and free of fear of anoxemia of the walls. The mobile vagina may then be drawn outward in the closing of the mucosal cutaneous wound, thereby preventing constriction of the vaginal orifice, even after a proper perineorrhaphy.

Cryosurgery[2, 4, 5] has proved effective in overcoming infection or controlling hemorrhage in the large, fungating infected malignant lesions so that surgery need not be delayed for any great length of time. It is also helpful in local recurrences, by destruction of the lesion, and is palliative in the neglected or inadequately treated or hopeless situation.

Although we cannot prevent cancer from occurring, we can prevent the invasive stage from developing and progressing by recognizing and removing the precursors or the incipient stage of the neoplasm.

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DR. RICHARD E. SYMMONDS, Rochester, Minnesota. I have enjoyed the opportunity to review Dr. Morley's complete manuscript, which he has summarized for us very succinctly. Most of the inadequately and haphazardly treated patients who have been excluded were seen, I would presume, in the very early years of the study that covers a 40 year period.

Over the last 25 years, more or less, the surgical management of this condition has been fairly well standardized at most medical centers to include a radical vulvectomy with en bloc-incontinuity groin dissections and with somewhat selective pelvic node dissection. This remains our treatment plan, except for the early microinvasive lesions. As with the reports of Way, Green, and others, the Michigan data appear to substantiate the need for this type of operative procedure.

Like Dr. Morley, in a sizeable series of cases we have yet to encounter a patient with *negative* groin nodes who has *positive* pelvic node metastasis. It appears that the pelvic node dissection can be limited rather safely to patients who are found to have positive groin nodes (preferably by frozen section study) and perhaps those patients who have clitoral-urethral involvement. When the pelvic nodes *were* involved by metastasis, very few patients have survived. If irradiation has anything to offer other than palliation, I presume it should be applied to the pelvis and lower aortic areas in those patients found to have pelvic node metastasis. I most certainly disagree with recent reports, referred to by Dr. Morley, suggesting that vulvar carcinoma can be better managed by radical vulvectomy followed by 4 to 5 weeks of irradiation to the groin—this to avoid the morbidity and prolonged hospitalization alleged to

occur with groin dissection. With a properly accomplished groin dissection, the incisions heal promptly with infrequent exceptions (usually in obese patients), serious complications are rare, and hospitalization should not exceed about 20 days.

What constitutes a properly accomplished groin dissection? I am no longer certain! In *direct contrast* to the technique advised in the manuscript, we cut rather thin skin flaps, the dissection proceeds from lateral to medial and deep to the fascia over the sartorius and adductor muscles rather than superficial, and we infrequently transplant sartorius muscle. We advocate suction drainage under the flaps as long as necessary and very early patient ambulation; Dr. Morley relies on pressure dressings and bed rest for several days. Despite these differences, our incidence of primary healing, duration of hospitalization, and the results obtained are quite comparable to those reported. This suggests that attention to good surgical principles—sharp expeditious dissection, careful handling of tissues, and good hemostasis—is much more important than some of the minutiae about which we differ.

In our North Central region, there appear to be three long-standing and persistent problems with this condition:

1. Delay in diagnosis, for which patient and physician are about equally responsible. We might ask, hopefully, if Dr. Morley's data indicate some improvement over the years, perhaps in relation to the size of the lesions encountered.

2. *Inadequate* primary therapy. Of the cases of vulvar carcinomas we see, 17 per cent are referred with recurrent malignancy following a previous local excision or "skimpy" vulvectomy. I presume Dr. Morley's experience has been similar—and how does one stage these patients?

3. Despite a presumably *adequate* primary operation, there is a significant incidence of local recurrence, most often in the perivaginal-periurethral area. Dr. Morley notes a recurrence rate of 33 per cent. How many of these were local recurrences and what are his recommendations for their prevention?

DR. JAMES H. NELSON, JR., Brooklyn, New York. Dr. Morley's complete paper carries a detailed description of the technique and preoperative care as he now performs the operation. This points up a problem with a retrospective study covering 40 years and particularly the past 40 years. He describes the use of prophylactic heparin therapy preoperatively and, in some patients, prophylactic antibiotics if indicated. Antibiotics, of course, were not available in 1935 and prophylactic heparin therapy is an approach used only in the past 5 or 6 years. A retrospective study covering 40 years has value in terms of determining the incidence of lymph node involvement where the regional nodes have been removed. A discussion of survival rates, postoperative complications, and operative deaths is much less meaningful, however, because of the many changes and improvements which have occurred over the last 40 years.

Indeed, it would be interesting to see the 5 year survival rates compared between cases treated from 1935 to 1940 and those treated from 1965 to 1970, as well as a comparison of the complications and operative deaths for those two periods. The foregoing is not a criticism peculiar to Dr. Morley's paper, but a growing concern of mine whenever papers are presented covering such a long retrospective period.

Dr. Morley has not made it clear as to when and why he decides to do a deep pelvic node dissection or not to do this procedure as part of the initial surgery. In our own institution we rarely do the deep pelvic node dissection as part of the initial radical vulvectomy and bilateral groin dissections. If the nodes in the groins are positive, then a deep pelvic node dissection is done 4 to 6 weeks later as a second-stage operation and is done by the transperitoneal route so that the paraaortic nodes can be examined and removed also, if necessary. I agree that the division of the inguinal ligament is unnecessary, and in my opinion, not the best approach.

Dr. Morley describes a two-team approach and seems to indicate that one team does the groin dissection on one side, and the second team does the opposite groin dissection. Our own two-team approach consists of one team doing both groins and a second team doing the vulvectomy simultaneously.

The use of pressure dressings is described, to eliminate dead space; they are left in place for 48 to 72 hours. I would point out to Dr. Morley that pressure dressings are apt to produce ischemia in skin edges that are already in jeopardy from lack of blood supply. We shifted several years ago at the suggestion of Stanley Way to suction drainage bilaterally which seems more physiologic. In this same regard Dr. Morley carries out the initial dressing change at 48 hours and the wound is left exposed and the patient gradually ambulated. Again, I would point out that 48 hours is too short a time if his intention is to treat the skin flaps as a graft. The tissues will continue to leak fluid and almost certainly raise the skin flaps for as long as 10 or 11 days postoperatively. Suction is frequently continued in our own patients for periods of approximately 10 days.

Dr. Morley states that after the operation, patients are kept in a semi-Fowler's position and allowed only "logrolling" for 48 hours. As I read this manuscript, and came upon the term "log rolling," I immediately conjured up a picture of a patient lying on her back imitating a man running on a log in the water that I used to see so commonly in newsreels. I hope Dr. Morley will define log rolling for us because it may be a very effective new Michigan method for avoiding thrombosis and embolism.

In the discussion of complications, the author has indicated that the complication encountered most frequently was wound necrosis or infection and

Table I. Incidence of lymph node metastases

Author	*No. of cases*	*No. with positve nodes*	*Per cent*
Green et al.	238	140	59
Way	84	44	52.3
Collins et al.	98	31	31.6
Nelson	70	27	38.5
Morley	194	64	33

secondary disruption. He has indicated that this outcome might well be anticipated since there is a significant amount of tissue damage and that the location of the wound makes it difficult to maintain a sterile field. He then points out the measures taken to prevent these problems. One is to consider the skin flaps as skin grafts and immobilize the area as much as possible, yet he has indicated that he mobilized the patient beginning at 48 hours postoperatively. We have noted a decided improvement in the incidence of wound breakdown by following Dr. Way's suggestion of immobilizing the patients for as long as 12 days. This is extremely difficult to do with the elderly patient and, in fact, we have abandoned this practice in patients 70 years of age or older, or in patients in the 60's who appear chronologically older. We have found if one attempts to immobilize the elderly patient she is apt to develop cerebral changes such as disorientation and amnesia. In the younger patient the protracted period of immobilization has no deleterious effect that we have been able to determine and does improve the healing of the skin flaps.

Surprisingly, Dr. Morley did not give actual statistics concerning complications. I realize that the title of the paper indicates the results of surgical treatment, but in my mind, complications are part of results. Dr. Morley has stated under the discussion of lymphedema that lymph nodes can never regenerate. We feel we have demonstrated quite clearly in the literature that, in fact, lymph nodes do regenerate after a pelvic node dissection.

Among the results reported the most noteworthy is that of the difference of survival rates between those patients who had no lymph node metastases vs. those with lymph node metastases. It is a most impressive difference, namely, 92.4 per cent corrected 5 year survival rate for those with no lymph node metastases and 38.7 per cent for those with lymph node metastases. Dr. Morley did not tell us what the patients with lymph node metastases died of, but it certainly raises the question as to what the physician should do in the way of further treatment of those patients who have lymph node metastases. It is also of interest that Dr. Morley has one of the lowest incidences of lymph nodes metastases of any series in the literature (Table I).

He also demonstrated very impressively that survival depended very little on whether it was a T_1 or T_2 lesion, but, as noted above, greatly depended on whether or not lymph node metastases were present. Of equal importance was Dr. Morley's finding that if a patient has either no lymph nodes involved or just one lymph node involved the prognosis is excellent, but with multiple nodes involved on one side or positive nodes bilaterally, prognosis is very markedly reduced.

One of the most important facts presented by Dr. Morley in this paper was that 18 per cent of the so-called early lesions, that is T1NOMO, had lymph node metastases. I hope those people constantly advocating conservative surgery will take note of this fact for they will condemn one out of five of their patients to die of this disease if they persist in advocating conservative surgery.

Dr. Morley's comments concerning surgical staging and the altering of the TNM system are pertinent. There is no question that the TNM system for vulva carcinoma as it is currently devised will not fly. One of the reasons the International Staging system in Gynecologic Oncology has been successful is because of its simplicity. Unless we simplify the vulva classification, I am afraid it will lose its usefulness.

In closing I shall ask Dr. Morley if he is planning any additional therapy for that group of patients with lymph node metastases?

DR. MORLEY (Closing). Fifty per cent of our cases were associated with some type of chronic vulvar dystrophy. If we can satisfactorily control the vulvar irritations then we can reduce the actual incidence of carcinoma of the vulva.

What about lymphangiography intraoperatively? We have not done this, but we should in the future, especially to determine the adequacy of the dissection.

Vaginal stenosis, anterior vaginal wall relaxation, and even stress urinary incontinence are delayed or remote complications. I do think that they can be treated satisfactorily by conventional methods. Time does not permit a detailed discussion of these procedures.

In regard to the T_2 lesions, it is interesting to note that in the 1940's the average greatest diameter of the T_2 lesion was 5.5 cm., and in the 1960's the average greatest diameter was 4.2 cm. I think that is significant and it certainly shows that we are seeing these lesions a little earlier now than previously.

I cannot tell you what percentage of our recurrences were distant metastases. I was primarily interested in the local recurrences; 60 per cent of them occurred in the vulvar area proper and 40 per cent of them occurred in the regional lymph node area. Our basic treatment for recurrence is simply wide local excision. We have not used cryosurgery to date.

Prophylactic anticoagulants are employed. We use minidose heparin for this purose.

It is obvious that this is a retrospective study and should be considered in this way. It does go back a long

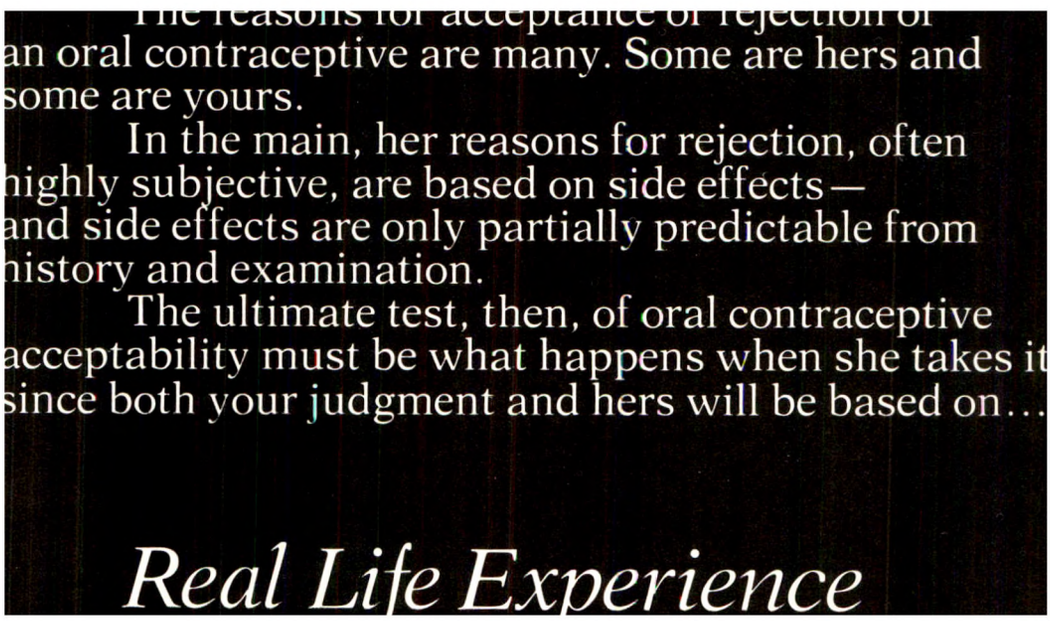
time and as a matter of fact I have only been there for the last 25 years of the study period. The study actually dates back to 1935, when a person you all know very well, Dr. Norman F. Miller, was Chairman of the Department at Michigan.

The 5 year survival rates in cases from 1935 to 1940 vs. 1965 to 1970 were determined but I cannot remember the exact figures. I can say, however, that early in the series more patients had to be treated with palliative vulvectomy or no therapy at all.

About pressure dressings . . . I have heard this before . . . that they do some bad! I use them primarily for hemostasis for the first 48 hours. We do a full-thickness dissection rather than a split-thickness (undermining) dissection and we think the pressure dressing serves to immobilize this tissue more effectively.

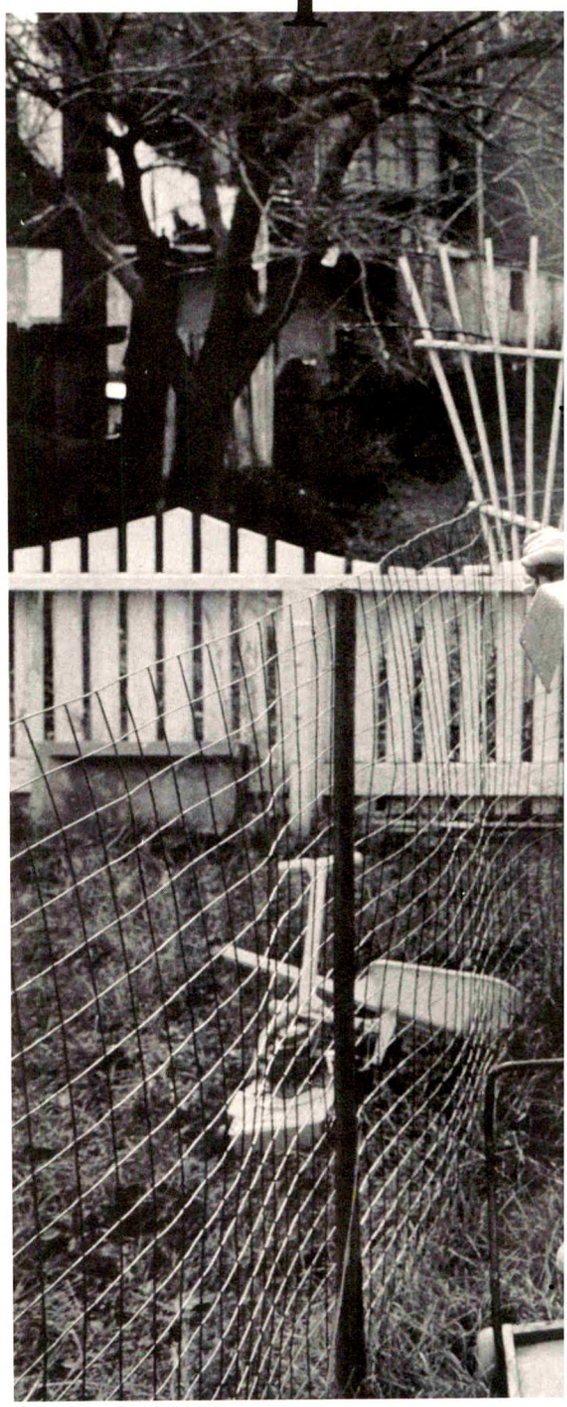
The immediate complications usually were wound infection and wound breakdown, but none had serious sequelae and all responded well to conservative management.

Lastly, about the rad
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study in the future.


The reasons for acceptance or rejection of
an oral contraceptive are many. Some are hers and
some are yours.
In the main, her reasons for rejection, often
highly subjective, are based on side effects—
and side effects are only partially predictable from
history and examination.
The ultimate test, then, of oral contraceptive
acceptability must be what happens when she takes it
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The woman profiled here is an actual ZORANE 1/50 user. However, not all women can be expected to have the same tolerance for side effects, should any occur. (Data on file, Lederle Laboratories)

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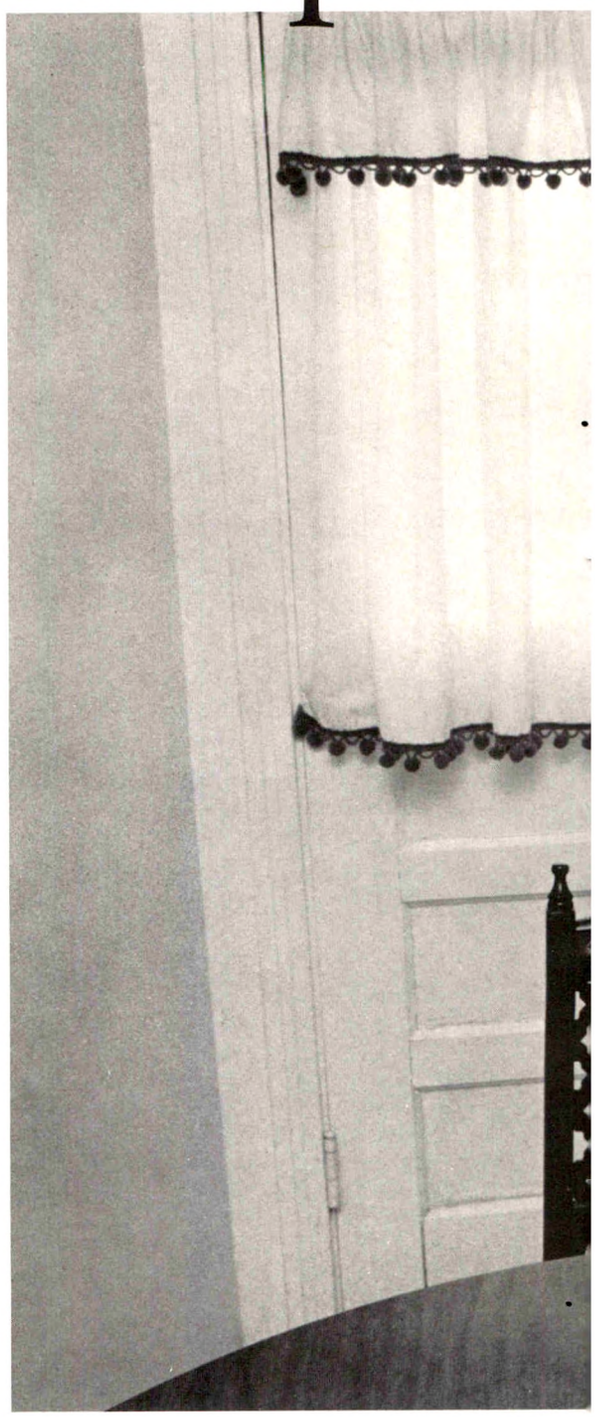
Judy Barrett, Housewife
Pittston, Pennsylvania
Age 22, married 6 years, 2 children
Cycle experience with Zorane 1/50: 8

he majority

"I don't seem to gain much weight with the pill and I don't get sick. I take it at night just before I go to bed... then I sleep right through. And I don't get sick."

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A Woman satisfied her oral contraceptive



Among the reasons patients remain satisfied: Withdrawal bleeding is usually regular and there is usually no undue discomfort

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From clinical studies: In a study population of 1156 women, 25,983 cycles, the incidence was 2.2% for amenorrhea and 2.0% for dysmenorrhea. Actual patient dropout rate* was 1.1% (13 subjects) for amenorrhea and 0.2% (2 subjects) for dysmenorrhea. Overall patient dropout rate was 14.4% due to all adverse reactions. Serious reactions, as well as minor adverse reactions, have been reported following the use of all oral contraceptives. Before prescribing, the complete product information should be consulted for *special note, contraindications, warnings, precautions* and *adverse reactions*. Thereafter, the physician should remain alert to the earliest manifestations of any serious disorder. Should any occur or be suspected, the oral contraceptive should be immediately discontinued.

The woman profiled here is an actual ZORANE 1/50 user. However, not all women can be expected to have the same tolerance for side effects, should any occur. (Data on file, Lederle Laboratories)

*Dropout rates are based on total patient population (1,156); incidence reflects only those patients who completed one or more cycles (956).

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Mrs. Linda McKinnon, Housewife
Nederland, Texas
Age 25, married 7 years, 2 children
Cycle experience with Zorane 1/50: 16

he majority

"You always know when you're going to start…I love knowing that… you can always plan…I think it's great. And, not having any cramps, too, because I used to have cramps."

From real life...

A Woman satisfiec her oral contraceptive



One reason for long-term patient satisfaction: Weight gain usually not a problem

ZORANE 1/50 is a highly effective and generally well-tolerated oral contraceptive—providing the estrogen content and progestin:estrogen ratio that appears to be highly acceptable to the majority of women.

From clinical studies: In a study population of 1156 women, 25,983 cycles, the incidence of weight gain was 0.4%. Actual patient dropout rate* due to weight gain was 2.0% (23 subjects). Overall patient dropout rate was 14.4% due to all adverse reactions. Serious reactions, as well as minor adverse reactions, have been reported following the use of all oral contraceptives. Before prescribing, the complete product information should be consulted for *special note, contraindications, warnings, precautions* and *adverse reactions*. Thereafter, the physician should remain alert to the earliest manifestations of any serious disorder. Should any occur or be suspected, the oral contraceptive should be immediately discontinued.

The woman profiled here is an actual ZORANE 1/50 user. However, not all women can be expected to have the same tolerance for side effects, should any occur. (Data on file, Lederle Laboratories)

*Dropout rates are based on total patient population (1,156); incidence reflects only those patients who completed one or more cycles (956).

Zorane® 1/50

Norethindrone Acetate, 1 mg and Ethinyl Estradiol, 50 mcg
Oral Contraceptive Tablets

Progestin/Estrogen content balanced fc

See last page of this advertisement for brief summary of prescribing information.

with



Alice Eichenberger, High School
Business Teacher, Louisville, Kentucky
Age 28, married 7 years, no children
Cycle experience with Zorane 1/50: 13

ne majority

"I've fluctuated between 115 and 120...and have had no appreciable weight gain...since I've been on Zorane."

Zorane® 1/50

Progestin/Estrogen content balanced for the majority

Norethindrone Acetate, 1 mg and Ethinyl Estradiol, 50 mcg

Oral Contraceptive Tablets

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Zorane 1/20 Norethindrone Acetate, 1 mg. and Ethinyl Estradiol, 20 mcg.

Special Note: Oral contraceptives have been marketed in the United States since 1960. Reported pregnancy rates vary from product to product. The effectiveness of sequential products appears to be somewhat lower than that of combination products. Both types provide almost completely effective contraception. The dropout rate for medical reasons, as observed in clinical trials, with ZORANE 1.5/30 appears to be the same as that of higher dose combination products. The dropout rate due to irregular bleeding was somewhat higher, but dropouts were equally split between irregular bleeding and other medical reasons attributable to the drug. (ZORANE 1/20 only: The effectiveness is somewhat lower than that of the higher dose combination products. Both provide almost completely effective contraception. Dropout rate for medical reasons appears to be somewhat higher than with higher dose combination products, due particularly to irregular bleeding.) An increased risk of thromboembolic disease associated with use of hormonal contraceptives has now been shown in studies conducted in both Great Britain and the United States. Other risks, such as those of elevated blood pressure, liver disease, reduced tolerance to carbohydrates, have not been quantitated with precision. Long-term administration of both natural and synthetic estrogens in subprimate animal species in multiples of the human dose increases the frequency for some animal carcinomas. These data cannot be transposed directly to man. Possible carcinogenicity due to the estrogens can neither be affirmed nor refuted at this time. Close clinical surveillance of all women taking oral contraceptives must be continued.

Indications: For control of conception.

Contraindications: Thrombophlebitis, thromboembolic disorders, cerebral apoplexy, or a past history of these conditions; markedly impaired liver function; known or suspected carcinoma of the breast; known or suspected estrogen-dependent neoplasia; undiagnosed abnormal genital bleeding; pregnancy.

Warnings: If early signs of thrombotic disorders (thrombophlebitis, cerebrovascular disorders, pulmonary embolism, retinal thrombosis) occur or are suspected, discontinue use of drug immediately. (Studies indicated a higher risk of these conditions with use of these drugs, not persisting after drug stoppage and not enhanced by long-term use.[1-4]) If sudden partial or complete loss of vision occurs, or if there is sudden onset of proptosis, diplopia or migraine; or if examination reveals papilledema or retinal vascular lesions, discontinue medication. Safety of use in pregnancy has not been established, therefore rule out pregnancy in any patient who has missed two consecutive periods before continuing contraceptive regimen; if prescribed schedule has not been adhered to, consider possibility of pregnancy with first missed period. A small fraction of hormonal agents has been identified in the milk of mothers receiving these drugs; long-range effect to the nursing infant has not yet been determined.

Precautions: Pretreatment and periodic physical examinations of breasts and pelvic organs should include Papanicolaou smear. (Estrogens have produced tumors, some malignant, in 5 species of subprimate animals.) If endocrine and liver function tests are abnormal in patients taking these drugs, repeat tests after drug withdrawal for 2 months. With use of these drugs, pre-existing uterine fibromyomata may increase in size; and some conditions (*i.e.*, epilepsy, migraine, asthma, cardiac or renal dysfunction) may be influenced by fluid retention; observe carefully for such. With use of ZORANE 1.5/30 or ZORANE 1/20, alteration in menstrual patterns may be induced. Since the amount and duration of flow and cycle length may vary, this must be considered in attempting to determine if cause of bleeding is organic. (ZORANE 1/50 only: In breakthrough bleeding or all cases of irregular bleeding per vaginam, keep in mind nonfunctional causes and take adequate diagnostic measures.) With history of psychic depression, discontinue drug if depression recurs to a serious degree. Possible influence of prolonged ZORANE therapy on pituitary, ovarian, adrenal, hepatic or uterine function awaits further study. A decrease in glucose tolerance has been seen in some patients on oral contraceptives; carefully observe diabetics on such therapy. Patient age is no absolute limiting factor, although these drugs may mask onset of climacteric. The pathologist should be advised of ZORANE therapy when relevant specimens are submitted. Susceptible women may have an increase in blood pressure following use of contraceptive steroids.

Adverse Reactions Observed in Patients Receiving Contraceptives: Statistically significant association demonstrated between use of oral contraceptives and: thrombophlebitis, pulmonary embolism, cerebral thrombosis. Suggestive evidence, neither confirmed nor refuted, between use and neuro-ocular lesions (*e.g.*, retinal thrombosis, optic neuritis). Known to occur: nausea, vomiting, G.I. symptoms (abdominal cramps, bloating), breakthrough bleeding, spotting, change in menstrual flow, amenorrhea during and after treatment, edema, chloasma or melasma, breast changes (tenderness, enlargement, secretion), weight change (increase or decrease), changes in cervical erosion and secretions, suppression of lactation when given immediately post-partum, cholestatic jaundice, migraine, allergic rash, rise in blood pressure in susceptible women, mental depression. Reported association with use, neither confirmed nor refuted: anovulation post treatment, premenstrual-like syndrome, changes in libido, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, fatigue, backache, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, itching. *May Alter Laboratory Results in:* hepatic function (increased sulfobromophthalein retention and other tests), coagulation tests (increase in prothrombin, Factors VII, VIII, IX, X), thyroid function (increase in PBI_1, and butanol extractable protein bound iodine, and decrease in T^3 uptake values), metyrapone test, pregnanediol determination.

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Effects of local anesthetic agents on the uterine vasculatures and myometrium

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Intravascular procaine, lidocaine, mepivacaine, and bupivacaine decrease blood flow to the placental and nonplacental vascular beds of gravid ewes by stimulating vasoconstriction and myometrial contractility. These effects appear to be direct ones since they are not affected by alpha-adrenergic blockade. Dose-response curves determined in nonpregnant ewes indicate that significant decreases in blood flow may occur at arterial blood concentrations encountered clinically. It is proposed that reduced placental blood flow is the cause of fetal bradycardia following paracervical block anesthesia. The implications of these findings in obstetric anesthesia are discussed.

DESPITE the widespread use of local anesthetic agents in obstetric practice, very little is known about the direct effects of such agents upon the myometrium and uterine vasculatures. In 1962, McGaughey and his associates[12] determined the in vitro effects of a variety of synthetic anesthetics on gravid and nongravid human myometrial strips. Of those agents frequently used today, tetracaine, lidocaine, procaine, and chlorprocaine all increased contraction amplitude and/or tonus. These investigators concluded "that the use of high dosages of these drugs for intravascular administration or for infiltration anesthesia should be employed by the obstetrician with circumspection until information concerning tissue concentrations of such agents existing clinically becomes available." To our knowledge, similar studies on myometrium have not been performed since that time and most concentration data concern that in blood rather than in tissues.

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It is generally believed that most local anesthetics relax vascular smooth muscle by a direct action and can produce vasodilatation. Mepivacaine and tetracaine are reported to have mixed or biphasic effects which are dose dependent and possibly dependent upon preexisting vascular tonus.[1] Since the number of such studies is limited, however, the effects are not certain, and no observations have been made on the uterine vasculature. It must be emphasized that we are speaking of the direct effects of local anesthetics, which may be significantly different from the cardiovascular effects of conduction anesthesia induced by local anesthetics—effects which have been studied extensively.

Recently, we observed unanticipated uterine responses when lidocaine was used to reduce cardiac irritability. Therefore the present study was undertaken to evaluate the effects of intravascularly administered local anesthetic agents upon the uterine vasculatures and myometrium.

Methods

In the four series of experiments performed, uterine blood flow (UBF) was measured by an electromagnetic flow probe, arterial (ABP) and venous (VBP) blood pressures from catheters placed in the abdominal aorta and a uterine collecting vein, and intrauterine pressure (IUP) from an open-ended catheter placed in the uterine cavity. Probes and catheters were implanted acutely or chronically, depending upon the particular experiment, and occlusion loops were implanted to

determine zero flow levels. The details of these techniques have been described previously.[6, 7]

Experiment 1. Eight gravid ewes between 110 and 145 gestation days were placed in the right lateral decubitus position, paralyzed with curare, intubated under topical anesthesia, and respired via a respiration pump with nitrous oxide–oxygen at gas mixtures and respiratory rates and volumes sufficient to maintain adequate anesthesia and normal arterial blood gas levels. Appropriate catheters and transducers were implanted acutely. Upon stabilization of all parameters, 5 ml. bolus injections of 20, 40, and 80 mg. of lidocaine-HCl or mepivacaine-HCl followed by 5 c.c. of 0.9 per cent sodium chloride were injected just above the aortic trifurcation and uterine responses were observed. Prior to the first drug injection, a control bolus of 5 c.c. of 0.9 per cent sodium chloride was given. All parameters were permitted to stabilize at control levels before each subsequent injection was given. Observed parameters were sampled at 15 second intervals during a 1 minute control period, at 5 to 20 second intervals during the period of maximum response, and at 1 minute intervals thereafter until control levels were re-established. UBF and perfusion pressure (ABP-VBP) were expressed as percentages of the mean control observations and IUP in absolute pressure units.

Experiment 2. Five gravid ewes between 69 and 107 gestation days were prepared acutely as described above. In addition a No. 7 Fr. single-curve catheter was inserted into the left ventricle via the carotid artery and an additional sampling catheter was placed in the femoral artery. Upon stabilization of all parameters a 5 ml. bolus of 80 mg. mepivacaine or 5 units of Pitocin was injected as described previously. During the control period prior to each injection, during the maximum response to mepivacaine-HCl and after 30 seconds of myometrial tetany induced by Pitocin, 1.0 to 1.5 c.c. of ^{141}Ce-, ^{51}Cr-, ^{85}St-, or ^{46}Sc-labeled 15 ± 5 μ microspheres diluted to 10 c.c. with 0.9 per cent NaCl were infused intraventricularly over a 15 to 30 second interval. Accurate placement of the cardiac catheter was confirmed by typical wide pressure tracings before and after each injection. Femoral arterial and uterine venous blood samples were withdrawn at a constant rate for the 15 seconds preceding, during, and 1 minute after each microsphere injection. Pressure and flow parameters were analyzed as described above.

At the completion of this protocol, the experimental preparation was sufficiently stable that clinically equieffective doses[4] of procaine or bupivacaine were administered either intra-arterially or intravenously to evaluate the effects of these agents as well as the influence of the route of administration on observed responses.

Upon termination of each experiment, the ewes were put to death and the uterus was dissected grossly into cervix, right and left cotyledons, and right and left myoendometrium. Individual tissues and blood samples were weighed, processed, and counted for radioactivity as described previously.[2] Total UBF was determined by the method of Makowski and associates[13] and individual tissue flows were calculated from their per cent of total organ radioactivity.

Experiment 3. Four nonpregnant oophorectomized ewes were implanted chronically. In addition, a catheter was implanted into a branch of the uterine artery for direct injection of test drugs after the method of Killam and associates.[9] Upon recovery from surgery, 1.0 μg per kilogram of 17β-estradiol dissolved in a solution of one part 100 per cent ethyl alcohol, 4 parts propylene glycol, and 5 parts distilled water was injected intra-arterially and UBF was observed until maximum levels were obtained. Thereafter, 10 minute uterine arterial infusions of mepivacaine HCl, lidocaine HCl, procaine HCl, or bupivacaine HCl were given at progressive concentrations from below threshold response to those causing a maximal response. Between infusions, parameters were observed until they returned to control levels. Infusion rates varied from 0.0764 to 1.91 c.c. per minute. Only one drug was tested on a given day and the sequence of drugs administered to each ewe was randomized. Mean UBF rates during a 3 minute control period and during a 3 minute maximum response period were determined by planimetry.

Experiment 4. Upon completion of Exp. 3, estradiol was given again on a subsequent day. When the maximum UBF response was observed, a 5 c.c. bolus of norepinephrine, 50 μg, or mepivacaine-HCl 40 mg. followed by 5 c.c. of 0.9 per cent NaCl was injected into the descending aorta and the responses were observed. Thereafter, phenoxybenzamine was infused over 30 minute periods to attain cumulative dose levels of 1.0, 3.0, 5.0, and 10.0 mg. per kilogram. At each level the norepinephrine bolus was repeated until no response occurred. Then the mepivacaine-HCl injection was repeated. Responses were determined as the maximum decrease in UBF following drug administration and were expressed as a per cent of a one minute control period.

Results

In all of the observations reported below, no significant changes from control levels in ABP or UBP occurred. Therefore, while uterine vascular responses

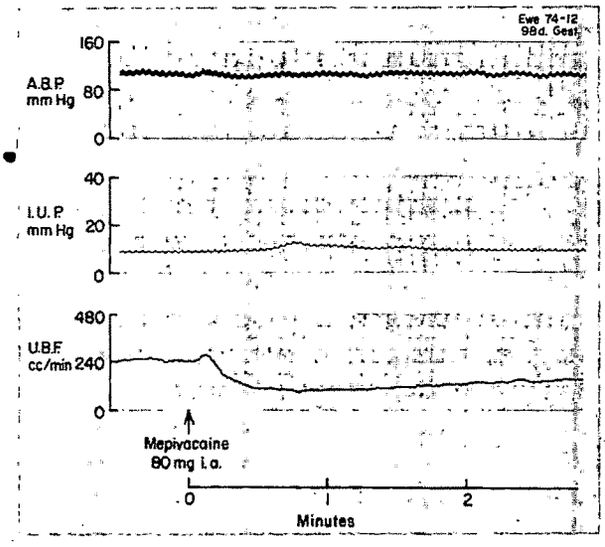


Fig. 1. Original tracing illustrating the effects of intravascular mepivacaine on the gravid uterus. Note the unchanged arterial blood pressure *(ABP)*, the minimal increase in intrauterine pressure *(IUP)*, and the marked decrease in uterine blood flow *(UBF)* apparently unrelated to the IUP change.

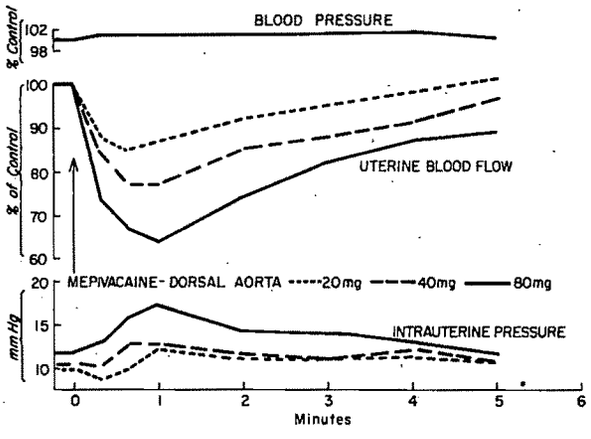


Fig. 2. Composite effects of increasing intravascular doses of mepivacaine on the uteri of third-trimester ewes. Note the progressive decrease in UBF with similar inverse changes in IUP.

have been described in terms of UBF changes, almost identical changes in uterine vascular conductance also occurred.

Experiment 1. An original tracing of a typical response to intra-arterial mepivacaine is shown in Fig. 1 and mean responses in eight ewes to increasing doses of mepivacaine are shown in Fig. 2. UBF decreased progressively with each dose increment, reaching a maximum fall to 64 per cent of control at the 80 mg. dose level. Significant decreases in UBF persisted for at

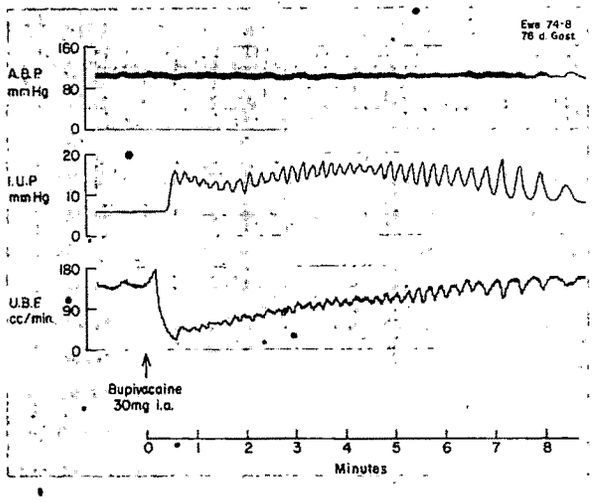


Fig. 3A. Effect of intravascular bupivacaine on IUP and UBF in a gravid ewe. Although IUP maintained a prolonged hypertonus, UBF changed independently.

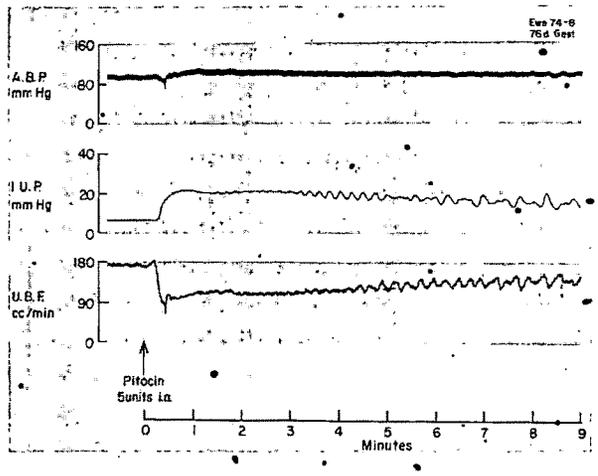


Fig. 3B. Effect of intravascular pitocin in the same ewe. UBF decreased less than in Fig. 3*A* despite a greater increase in IUP and varied inversely with the IUP changes.

least 2 minutes at the 40 and 80 mg. dose levels. Similar decrements in UBF occurred after lidocaine administration (Table I), although a wide response variation occurred with both agents. Simultaneously, mean IUP increased, peaking at response times similar to the maximum decreases in UBF. While the myometrial contractility generally increased in relation to the dose of drug, a wide range of responses also occurred, occasionally with no discernible changes in IUP, associated with a moderate fall in UBF. Since the effect of myometrial contractility on UBF is difficult to quantitate in the ewe, the relative effects of uterine vasoconstriction and myometrial contractions on the UBF response were impossible to evaluate.

Table I. Effects of intra-arterial anesthetics on gravid uterine blood flow

	Time after injection (sec.)						
	20	*40*	*60*	*120*	*180*	*240*	*300*
Lidocaine 20 mg.	90 ± 4.3*	90 ± 6.1	90 ± 8.9	97 ± 4.2	100 ± 4.0	104 ± 6.9	111 ± 8.1
Mepivacaine 20 mg.	88 ± 2.7	85 ± 8.2	87 ± 8.9	92 ± 7.2	95 ± 6.0	98 ± 6.2	101 ± 3.5
Lidocaine 40 mg.	86 ± 6.4	77 ± 5.9	78 ± 4.0	84 ± 5.8	92 ± 7.9	92 ± 6.7	94 ± 8.2
Mepivacaine 40 mg.	84 ± 7.1	77 ± 6.6	77 ± 6.6	85 ± 6.0	88 ± 6.0	91 ± 6.0	96 ± 5.2
Lidocaine 80 mg.	86 ± 6.4	73 ± 6.0	68 ± 6.1	72 ± 5.1	84 ± 7.4	90 ± 7.6	96 ± 9.8
Mepivacaine 80 mg.	74 ± 7.8	67 ± 8.5	64 ± 10.0	74 ± 12.2	82 ± 11.3	87 ± 10.4	89 ± 7.2

*Mean per cent of control UBF ± 1.0 S.D.

Table II. Effects of intra-arterial mepivacaine and pitocin on total uterine (UBF_E), placental (PBF), and nonplacental (NPBF) blood flows

	UBF_E	*PBF**	*NPBF**	*ΔIUP† (mm. Hg)*
Mepivacaine 80 mg.	50.8 ± 8.0	47.8 ± 7.3	65.0 ± 9.0	+2.7
		$P < 0.05$	$P < 0.02$	
Pitocin .5 units	74.8 ± 3.9	68.2 ± 2.2	102 ± 4.8	+10.5

*Per cent of control ± S.E.
†Intrauterine pressure change.

Experiment 2. Uterine responses to mepivacaine were similar to those described above. After the Pitocin bolus, ovine myometrium contracted tetanically, thereafter to devolve into cyclic uterine contractions of progressively decreasing tonus and intensity (Fig. 3*B*).

In these experiments, unilateral or total UBF determined by the microsphere technique did not correlate with relative changes in UBF determined by the flowmeter. However, uterine venous radioactivity was negligible, indicating almost complete entrapment of microspheres within the uterine vasculatures. It was concluded on the basis of previous observations[2] that the rapid injection of microspheres required by this experiment precluded sufficiently representative arterial microsphere contents to permit accurate UBF determinations by this method. Previously, unilateral UBF determinations by the flowmeter technique have been shown to be almost exclusively representative of unilateral UBF in the ewe.[8] It has also been shown that rapid microsphere injections will accurately reflect flow distribution within the uterus when accurate determinations of total UBF by the same method have been compromised.[2] Therefore, the proportionate distribution of UBF in the placental and nonplacental vascular beds of the uterine horn ipsilateral to the flow probe were determined and, by cross-multiplication with the flowmeter-determined UBF, blood flows to the two uterine vasculatures were derived. These results are shown in Table II. Mepivacaine caused placental and nonplacental UBF to decrease to 47.8 and 65.0 per cent of control values, respectively, during a mean increase in IUP of 2.7 mm. Hg. Thirty to 70 seconds after the onset of myometrial tetany induced by pitocin, placental blood flow decreased to 68.2 per cent of control levels and nonplacental flow was essentially unchanged while IUP increased 10.5 mm. Hg, four times that observed after the mepivacaine injection. The relative changes in placental and nonplacental blood flows after the two stimuli were statistically significant, as determined by the paired Student's t test at the <0.05 and <0.02 levels, respectively.

The responses determined above are supported by representative recordings of random observations obtained subsequently. In Fig. 3*A*, responses to a 30 mg. dose of bupivacaine, clinically equieffective to that of mepivacaine 80 mg. and to a bolus of Pitocin, are shown. Bupivacaine decreased UBF to less than 25 per cent of control levels when IUP increased 9 mm. Hg (Fig. 3*A*). Thereafter, UBF gradually increased to control levels despite a sustained increase in myometrial tonus. After Pitocin, UBF decreased to 50 per cent of control levels when IUP increased 15 mm. Hg. Thereafter, UBF inversely followed changes in IUP.

Following a clinically equieffective dose of procaine (Fig. 4), UBF decreased maximally prior to any change in IUP. Thereafter, effects of myometrial contractility and uterine vasoconstriction were not separable.

Experiment 3. Evaluation of responses was complicated by two factors. First, it was impossible to correlate IUP changes with UBF responses (Fig. 5*A*). Such lack of correlation in the nonpregnant ewe when the open-ended catheter technique was used as a measure of myometrial activity has been observed previously. Presumably, local IUP at the catheter end does not represent in bicornuate uteri the cumulative myometrial activity influencing UBF. Therefore, monitoring of IUP was abandoned and myometrial activity was

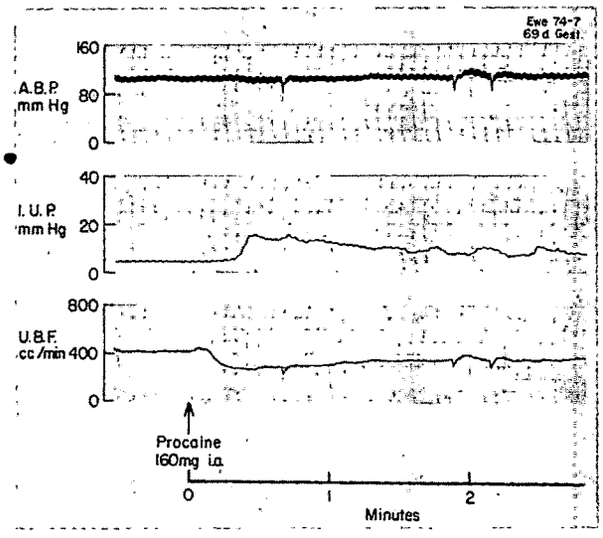


Fig. 4. Effects of intravascular procaine on the gravid ovine uterus. The decrease in UBF preceded the rise in IUP, indicating significant intrinsic uterine vasoconstriction.

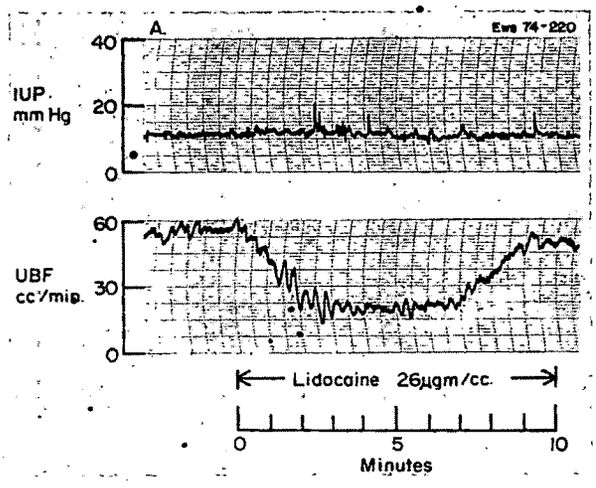


Fig. 5A. Effect of intravascular lidocaine on the nonpregnant ovine uterus. Note the transient cyclic changes in UBF and the lack of correlation between IUP and UBF patterns.

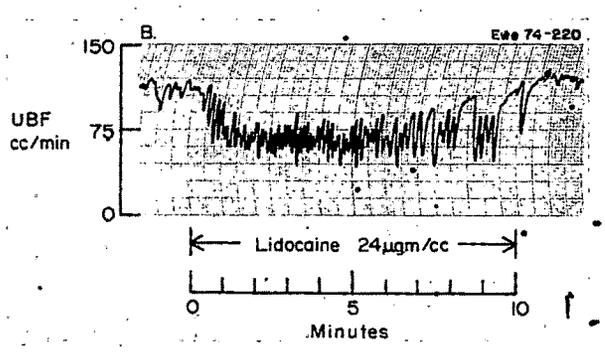


Fig. 5B. Effect of intravascular lidocaine on the same ewe as in Fig. 5*A* but on a different day. The rapid cyclic changes in the UBF pattern differed markedly from those in Fig. 5*A* although ABP was constant.

presumed from the UBF patterns. Essentially flat UBF control patterns changing to cyclic patterns during the response period (Figs. 5*A* and 5*B*) were interpreted as indicating the onset of cyclic increases in myometrial activity. When the response patterns were also flat, tetanic myometrial activity may have been occurring, but such a response could not be differentiated from uterine vasoconstriction alone. Second, there was a tendency for falls in UBF to recover toward control levels while the test drug was still being given. This was not a constant observation with respect to a particular drug or animal and we could not differentiate a tachyphylactic effect from an autoregulatory one. Therefore responses were interpreted with respect to the maximum consecutive 3 minute decrease in UBF during the time of drug administration, recognizing that the relative influences of multiple etiologic factors could not be assessed.

Regression lines describing the dose-response relationship of the four drugs tested are shown in Figs. 6*A* to 6*D* with individual dose-response observations to emphasize the wide variations which occurred. This was particularly true with lidocaine, where the correlation coefficient was 0.44 and a wide range of threshold response doses was observed. The four dose-response curves are depicted in Fig. 7 for comparison. The intercept for bupivicaine was significantly different ($P < 0.05$) from that for procaine and mepivacaine but not for lidocaine. Thus the threshold concentration of bupivacaine above which UBF decreased was about 1.0 μg per cubic centimeter, whereas that for the other three local anesthetics approximated 3 to 4 μg per cubic centimeter. The slope of the lidocaine regression line was shallower than that observed with the three other drugs. The regression coefficient for lidocaine was significantly different ($P < 0.05$) only from that of bupivacaine.

Experiment 4. As depicted in Fig. 8, uterine vasoconstriction following norepinephrine stimulation was blocked at the level of 10.0 mg. per kilogram of phenoxybenzamine. The decrease in UBF after mepivacaine injection remained unchanged despite complete alpha-adrenergic blockade.

Comment

The present observations demonstrate that local anesthetic agents administered intravascularly can cause significant reductions in gravid and nonpregnant UBF. This effect occurs as a result of vasoconstriction in both placental and nonplacental circulations and as a

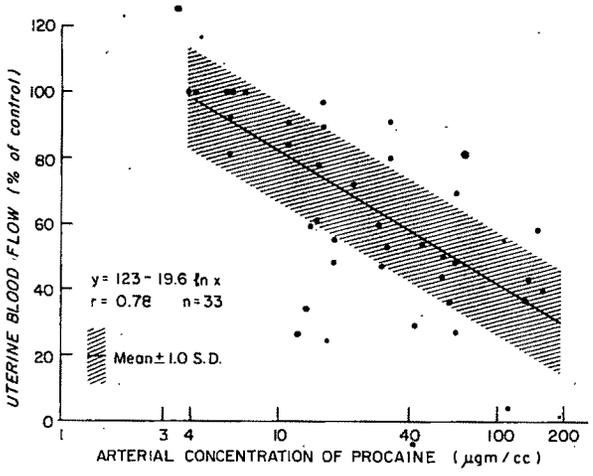


Fig. 6A. Dose-response curve in nonpregnant ewes with procaine, b) lidocaine, c) mepivacaine and d) bupivacaine shown with individual observations (•) to illustrate the wide range of responses.

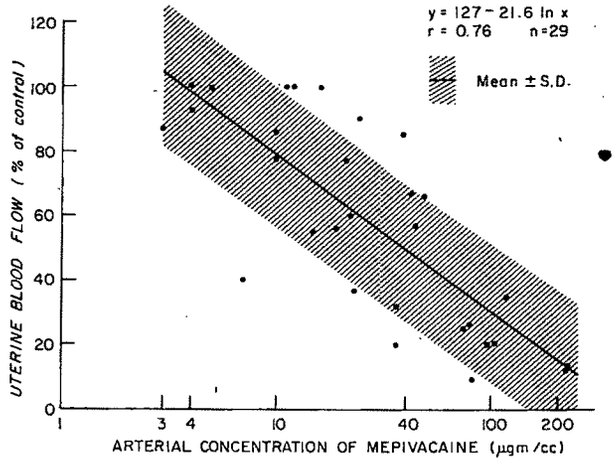


Fig. 6C. Dose-response curve in nonpregnant ewes with mepivacaine. Legend as in Fig. 6*A*.

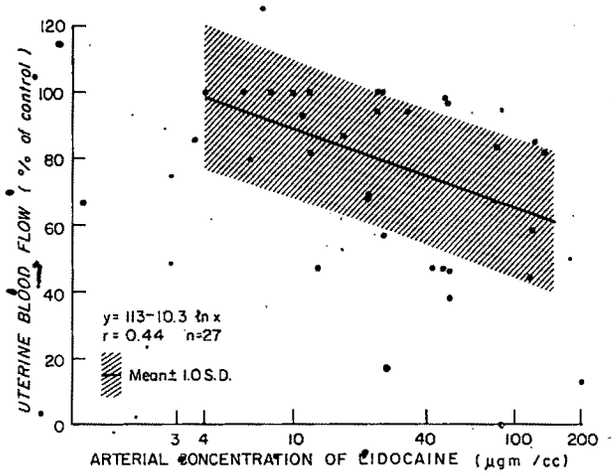


Fig. 6B. Dose-response curve in nonpregnant ewes with lidocaine. Legend as in Fig. 6*A*.

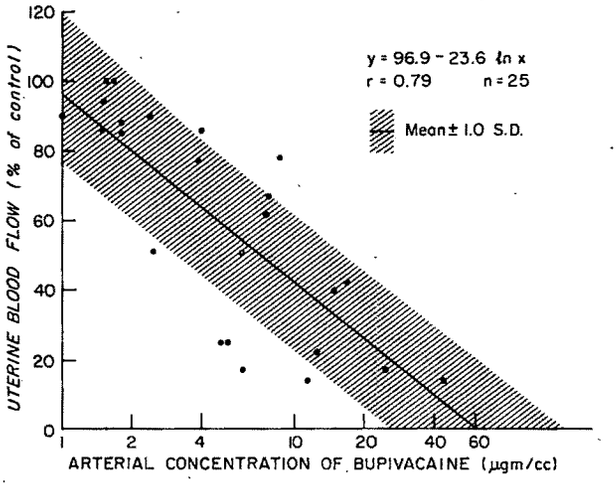


Fig. 6D. Dose-response curve in nonpregnant ewes with bupivacaine. Legend as in Fig. 6*A*.

result of increased myometrial activity, although the former mechanism seems the dominant one, since UBF reductions were observed in the absence of demonstrable IUP increases and exceeded those anticipated from IUP increases alone. The effect would appear to be a direct one, since it was not affected by alpha-adrenergic blockade. In the nonpregnant uterus maximally dilated with estradiol, threshold concentrations above which UBF reductions occurred were 1.0 μg per cubic centimeter with bupivacaine and between 3 and 4 μg per cubic centimeter with procaine, lidocaine, and mepivacaine. The differences between the bupivacaine threshold and those of lidocaine and mepivacaine are consistent with clinically equieffective doses of the agents. On the same basis, the procaine threshold would have been expected to be 10 μg per cubic centimeter so that it would appear that clinically equieffective dosages of the various local anesthetics may not be a valid prognosticator of the uterine response. While significant differences in dose effectiveness and the slopes of the dose-response curves were demonstrable from the present data, it must be emphasized that wide response variations occurred, particularly with lidocaine, and we do not believe that any firm conclusions should be drawn with respect to the relative clinical safety in obstetrics of any of the tested agents. This is especially true since dose-response observations have not been made during

pregnancy nor have the effects of local anesthetics on primate myometrium in vivo been tested. In the present studies, placental blood flow decreased more than nonplacental flow. While the degree of nonplacental vasoconstriction may have been partially offset by vasodilation in response to the increase in myometrial activity, similar offsetting responses would be expected to occur in the nonpregnant uterus so that one would anticipate similar if not lower threshold dose levels in the placental vasculature. In the ewe, myometrial responses typically are less than those reported in primates and the dynamics of the control of placental blood flow are different. Therefore one might expect a greater effect of local anesthetics on placental blood flow in the primate than have been presently reported.

Irrespective of these constraining considerations, local anesthetic agents appear to cause a significant reduction in UBF. In the present studies, a 25 per cent mean reduction in UBF was caused by the following uterine arterial concentrations of local anesthetics: bupivacaine, 2.5 μg per cubic centimeter; mepivacaine, 12 μg per cubic centimeter; procaine, 15 μg per cubic centimeter; and lidocaine, 40 μg per cubic centimeter. These concentrations are generally similar to those which might occur following paracervical block anesthesia or after accidental intravenous administration complicating epidural anesthesia.

It is of significance that nonplacental (myoendometrial) blood flow rates did not change during pitocin-induced myometrial tetany (Table II). These results confirm the observations of Lees and his associates[10] in the primate uterus and indicate that the myometrial vasculature dilates in response to myometrial activity to an extent that overrides the extrinsically induced uterine vasoconstriction from the same increase in myometrial contractility. During myometrial diastole, a transient upsurge of blood flow would be expected consistent with previous observations that the myoendometrial circulation possesses autoregulatory capabilities.

The cause of fetal bradycardia complicating paracervical block anesthesia during labor has been studied extensively. There is general consensus that the fetal response is related to the dose of local anesthetic administered. In such cases, the fetal scalp blood levels of local anesthetics usually exceed maternal peripheral blood levels,[3] indicating that at some time uterine arterial concentrations of these agents exceeded those in the fetus. When the fetus is delivered during or immediately following the bradycardic episode, Apgar scores are low and fetal

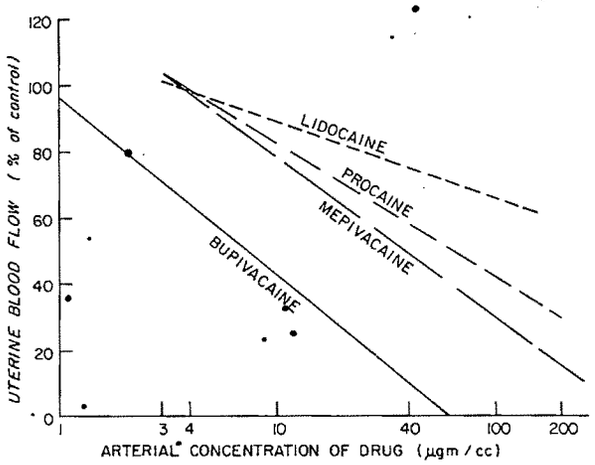


Fig. 7. Dose-response curves with four local anesthetics in nonpregnant ewes illustrating comparative responses. The threshold dose of bupivacaine was significantly different from those of the other agents. Although the lidocaine slope was different from that of bupivacaine, the wide response variations preclude clinical implications.

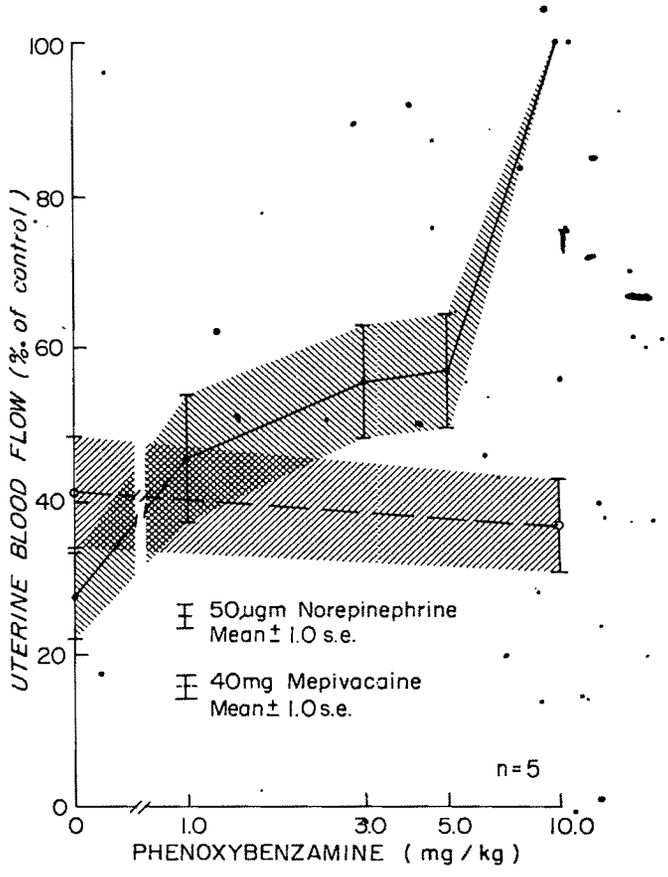


Fig. 8. Effects of norepinephrine and mepivacaine on the nonpregnant uterine vasculature at progressive levels of alpha-adrenergic blockade. The unchanged mepivacaine response indicates a direct vascular effect.

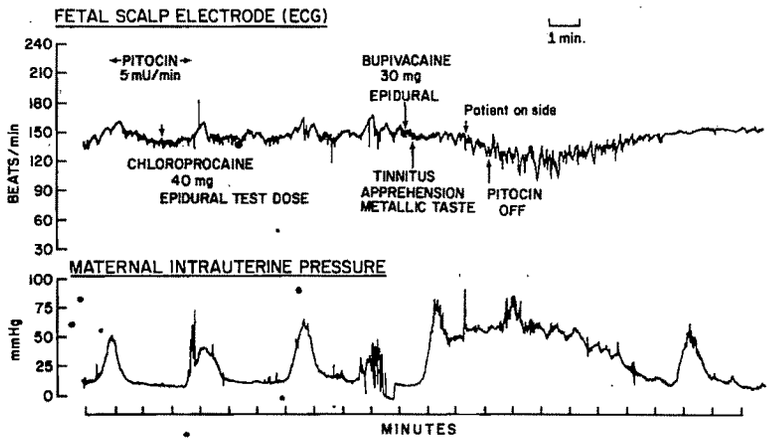


Fig. 9A. IUP and fetal heart range changes in a laboring woman after an intravenous injection of bupivacaine.

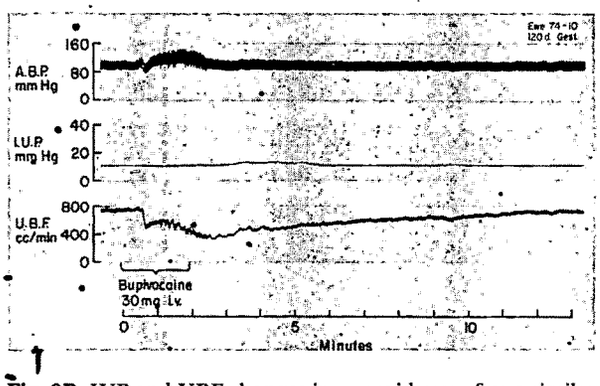


Fig. 9B. IUP and UBF changes in a gravid ewe after a similar injection of bupivacaine. See text for an interpretation of these responses.

depression has been attributed to high newborn caine levels. The observations of Freeman and his associates[5] belie such conclusions, however, since toxic levels of caines in the fetus cause increased cardiac conduction times and are associated with bradycardia only terminally. In contrast, the bradycardia following paracervical block is typified by a decreased P-R interval, variably inverted or absent P waves, and an unchanged QRS interval occurring at the time of the maximal bradycardic response. While fetal hypoxia has not been demonstrated during such episodes, observed decreases in pH levels and increases in base deficit strongly support a hypoxic etiology for the fetal heart rate changes. Associated observations include a variable incidence of increased myometrial activity and a higher frequency of bradycardia in premature, high-risk, and primigravid pregnancies. The present studies may explain the above-noted observations. It would appear that the doses of local anesthetics necessary to decrease UBF may occur following administration of the paracervical block. In this procedure, local anesthetics are injected blindly so that high uterine arterial concentrations may occur as a result of the injection site or from rapid penetrance of the local anesthetic across uterine arterial walls. Upon the onset of decreased UBF, the fetus may or may not develop hypoxic bradycardia, depending upon the prevailing adequacy of the uteroplacental unit. This proposed mechanism for the etiology of paracervical block—induced fetal bradycardia accounts for most if not all of the reported characteristics of the response.

If this mechanism is true, one must then consider the place of paracervical block anesthesia in obstetric practice. Since the placement and arterial uptake of local anesthetics as well as their effects upon the uterus are variable and therefore unpredictable, paracervical block anesthesia appears contraindicated in any pregnancy where adequacy of the uteroplacental unit is thought to be compromised. Similarly, if fetal bradycardia follows such anesthesia, uteroplacental adequacy should be suspect and the subsequent labor should be monitored closely. Upon the onset of bradycardia, the fetus is obviously distressed but, since the stressful conditions usually abate promptly, conservative management is indicated. In those unusual circumstances when bradycardia persists beyond 10 to 15 minutes, aggressive intervention by prompt atraumatic delivery is indicated. In such circumstances, an unusual degree of uterine vasoconstriction has occurred and/or a borderline adequacy of the uteroplacental unit has been identified. These conclusions must also be interpreted in the light of the obvious effectiveness, availability, and maternal safety of this form of anesthesia. Given an apparently normal parturient, paracervical block anesthesia has been demonstrated to be a safe anesthetic modality. Efforts should be made to reduce the dose of the anesthetic

agent to its lowest effective level, however, and the suggestion that contralateral administrations be spaced 10 to 15 minutes apart merits serious consideration.

Currently, epidural block anesthesia has become very popular and this anesthetic modality is used frequently in high-risk pregnancies. If maternal hypotension is prevented and the parturient is kept in a lateral decubitus position, adverse effects upon the fetus would seem minimal. Although systemic absorption of lidocaine and mepivacaine from the epidural space results in minimal maternal blood levels[11, 15] even with respect to the present observations, bupivacaine blood levels may approach threshold uterine vasoconstrictive levels.[14] Furthermore, accidental intravenous injections of the entire epidural dose of local anesthetic may occur via a misplaced epidural catheter or a catheter that has wandered into an epidural vein. If one assumes a maternal blood volume of 5,000 c.c. and instantaneous equilibration of the local anesthetic, a 100 mg. intravenous dose of mepivacaine or a 30 mg. dose of bupivacaine would result in arterial blood concentrations reaching the uterus of 20 and 6 μg per cubic centimeters, respectively—concentrations which may decrease UBF 25 to 50 per cent. Fig. 9*A* illustrates human myometrial and fetal heart rate responses following such an occurrence during labor. After a test dose of chlorprocaine, a 30 mg. dose of bupivacaine was administered. Within 15 seconds, the patient experienced typical symptoms of an intravenous injection; tinnitus, apprehension, and a metallic taste. Almost immediately, a sustained increase in IUP occurred which began to abate 5 minutes later. After 1 to 2 minutes, a modest decrease in the fetal heart rate occurred which began to increase coincident with or slightly before the decrease in IUP. To facilitate theoretical interpretation of this response, an identical dose of bupivacaine was injected intravenously to a gravid ewe over a 2 minute period, that time usually necessary to deliver the clinical dose through a 24 gauge needle connected to a 20 gauge epidural catheter (Fig. 9*B*). In this animal, there was a transient increase in ABP, the only time such a response occurred in the present series of experiments. During the phase of hypertension and thereafter, uterine conductance decreased approximately 50 per cent. Three minutes after the bupivacaine injection was started, UBF began a gradual climb toward control levels which were reached after 13 minutes. If one assumes that a similar UBF response occurred in the human illustration, the fetal bradycardia probably resulted from a reduction in UBF secondary to a combination of myometrial hyperactivity and placental vasoconstriction. Since the fetal bradycardia began to abate prior to or coincident with the fall in IUP, it is likely that placental vasoconstriction ameliorated before the IUP reduction, thus permitting UBF to increase to levels adequate for normal fetal oxygenation and recovery from hypoxic cardiac depression. In this illustration, the uteroplacental unit must have been most adequate to withstand such a theoretical decrease in UBF with only a modest slowing of the fetal heart rate. Were similar circumstances to occur in a high-risk pregnancy with borderline uteroplacental adequacy, severe fetal hypoxia and possibly fetal death may have occurred. Therefore, it would seem appropriate that the use of epidural anesthesia for high-risk pregnancies should be re-evaluated and that improved methods should be developed to avoid intravenous injections of local anesthetics.

It is even more difficult to postulate the potential effects of techniques such as paravertebral block and pudendal block anesthesia upon the fetus. At present we have no reason to believe that such techniques pose a threat to the fetus. However, increased attention must be placed upon minimal dosage and avoidance of intravascular injections when any local anesthetic technique is used in obstetric practice.

We thank John I. Fishburne, M.D., for permitting us to use the labor record used in Fig. 9 and A. Leonard Rhyne, Ph.D., for his help in compiling the statistical data.

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Discussion

DR. CHARLES E. FLOWER, Birmingham, Alabama. We are fortunate in this organization to have a distinguished group of "sheep herders" who provide us with food for thought and the warmth of intellectual stimulation. This study provides us with both.

It has always been painful for me to read or hear derogatory remarks about paracervical anesthesia. It is such an attractive idea! (1.) Paracervical anesthesia allows the injection of a local anesthetic away from the major constituents of the nervous system. (2.) It does not cause sympathetic or parasympathetic blockade. (3.) It is not associated with changes in blood pressure or cardiac return. (4.) It is not associated with the retardation of labor or increase in the incidence of pitocin stimulation.

This is in contrast to epidural anesthesia. (1.) Epidural anesthesia may cause a significant paralysis of the sympathetic and parasympathetic nervous systems. (2.) It may be associated with changes in homeostasis involving blood pressure and cardiac return. (3.) It is associated with a 20 to 50 per cent incidence of pitocin stimulation. In epidural anesthesia, the patient may be paralyzed and subjected to an artificial labor.

But wishing will not make it so. Asling and co-workers[1] have shown that infants who develop bradycardia following paracervical anesthesia have higher caine levels than do those infants who receive paracervical anesthesia without bradycardia. Teramo and Widholm[2] have shown that infants who have bradycardia may have a base deficit as well as a fall in pH. These are significant findings. But this study by Dr. Greiss and his colleagues indicates even more important alterations in fetal physiology, namely, that fetal bradycardia is associated with fetal hypoxia secondary to a reduction in uterine blood flow and may be associated with uterine hypertonus. Moreover, this study confirms the results reported by Freeman and co-workers,[3] who found that the electrocardiagrams of infants who develop bradycardia following paracervical anesthesia have changes consistent with hypoxia, and that local anesthetics injected into anencephalic infants induced electrocardiographic changes that are different than those induced with paracervical anesthesia.

Dr. Greiss was also able to demonstrate in his ovine patient what Dr. Fishburne observed in a primate patient, i.e., the intravenous injection of a local anesthetic may be associated with hypertonus and bradycardia that are most likely related to a reduction in uterine blood flow.

We must not abandon paracervical anesthesia, however. Understanding the cause and effect of an agent and its potential dangers may allow us to use the agent more effectively and safely.

Since the cause of the bradycardia is related to a reduction in uterine blood flow and hypertonus, it is no longer necessary for us to look for a modification in the caine nucleus that would allow the fetus to metabolize the agent more rapidly. We must develop a method of introducing paracervical anesthesia which will avoid the blind injection which is so frequently associated with an intravascular injection or the deposition of the material in the broad ligament which will allow its rapid uptake by the uterine vessels. This can be accomplished by using better vaginal exposure so that, under direct vision, the anesthetic can be deposited in a "wheal" beneath the vagina. We must reduce the dosage of the local anesthetic by using 0.25 per cent bupivacaine or 0.5 per cent mepivacaine with 1 to 200,000 epinephrine. This should provide safe anesthesia for 1½ to 3 hours. We should heed Dr. Greiss' advice and introduce the agent on one side at a time with a delay of at least 15 minutes between injections.

A new evaluation of paracervical anesthesia with these modifications should begin immediately. Until these techniques and this dosage schedule have been appropriately evaluated, however, we should discontinue the use of paracervical anesthesia in the case of small premature infants, patients with vascular disease and a possible reduction in uterine blood flow, in growth-retarded infants, in patients with obstetric hemorrhage, and in diabetics. These patients may have a reduction in blood flow and insufficient "placental

reserve" to prevent a damaging hypoxia from the vasoconstricting effects of the local anesthetic injected paracervically.

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DR. FREDERICK ZUSPAN, Columbus, Ohio. I would like to go back and have a little recall of 13 years ago in 1962 in Dr. Greiss' laboratory when we argued and talked about the importance of investigating the paracervical block in pregnant ewes, and really thought I was going to hear about that today.

The concern I have in the data presented is the extrapolation of data from sheep to human beings, even though most of the models that Dr. Greiss has shown in the past have lent themselves in a very valid way to human therapy and human investigation. I would like to share with you an investigation that has happened in the last 2½ years when I was at the University of Chicago. The work of Dr. Luis Cibils underscores another parameter in investigating uterine arteries and veins in a muscle bath to caine derivatives. The conclusions that he reached are identical to what Dr. Greiss has presented which is the response of the caine to cause vessel constriction, which in turn means that you have a decrease in uterine blood flow. Dr. Flowers' points are extraordinarily well taken, because paracervical block technique is indeed very important. There are several factors that involve the occurrence of bradycardia: (1) the location of the tip of the needle, and the bevel of the needle, whether it is directed toward the uterus or away, (2) second, the depth of the injections, and so technique (as often is the case in our specialty) is indeed important as to whether or not you are going to have a high or low occurrence of bradycardia. We can say with some degree of certainty now, and I would encourage Dr. Greiss to go back and really do paracervical blocks in pregnant ewes as we discussed 13 years ago, to work this out a little bit more, but the response will probably be vasoconstriction. I would hope that we would not abandon paracervical blocks, and I share Dr. Flowers' concern that this is indeed an elegant, simple, and easy way of achieving anesthesia and analgesia, and if we wave the flag too hard in the opposite direction, we may end up losing something that seems good now.

DR. WILLIAM N. SPELLACY, Gainesville, Florida. I want to congratulate Dr. Greiss on an excellent study. Earlier his pioneering work demonstrated that uterine blood flow could be increased by the administration of estrogens. Have you attempted to correlate the hormonal status of any animals with the response that you obtained by giving your caine derivatives? Second, would you care to speculate on whether or not the high-risk pregnancy and the preterm pregnancy fetal bradycardia responses to the paracervical block might be related to the hormonal milieu in those gestations?

DR. EDGAR MAKOWSKI, Denver, Colorado. I rise to congratulate Dr. Greiss on his elegant work. However, there are two questions. Since the experimental model utilized could have sustained a systemic effect from the injection of the local anesthetic, did you measure the cardiac output in any of these animals or note any changes in pulse rate? Second, did you observe any shunting across the uterus following the administration of the local anesthetic?

DR. GREISS (Closing). A number of years ago, Dr. Zuspan, others in the audience and I talked about giving a paracervical block to a ewe. I have tried this and believe it impossible due to anatomic differences. However, I think the question about using the ewe as an experimental model is very pertinent, particularly since myometrial responses are so hard to evaluate. We did not do the dose-response curves in pregnant ewes since the pregnancy season had run out. These experiments are planned for the coming year. Dr. Guy Harbert at Charlottesville has a very nice chronic primate model capable of measuring the same parameters as presently reported. His observations in a few preliminary experiments suggest that we will see the same qualitative responses in the primate as in the ewe. My own feeling is that a greater myometrial response will occur in the rhesus monkey from caine stimulation with a possibly greater uterine vasoconstriction.

Dr. Spellacy asked about the possible effect of the hormonal background on the caine response. The nonpregnant ewes were castrate and were given estradiol to cause maximal vasodilatation similar to that which is present during pregnancy. The pregnant ewes presumably had a normal hormonal background for their stage of pregnancy. While we have not used supplemental progesterone, I would doubt that significant differences in the caine response would occur.

Dr. Makowski, there were minimal changes in blood pressure or in pulse rate and we did not measure cardiac output. With respect to shunting, we did measure uterine venous content of microspheres and found almost no shunting with or without caines.

Elective cesarean hysterectomy: A 5 year comparison with cesarean section

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Elective cesarean sections performed on the obstetric service at the University of Arkansas Medical Center were reviewed for the period January 1, 1970, through December 31, 1974. The purpose of the review was to compare operative and postoperative complications of cesarean section, cesarean section and tubal ligation, and cesarean section and elective hysterectomy. A total of 1,255 cesarean sections were performed of which 207 (17 per cent) were associated with tubal ligation and 242 (18 per cent) with hysterectomy. Elective cesarean hysterectomies were performed for elective sterilization (68 per cent), for medically indicated sterilizations (11 per cent), or for definitive treatment of uterine pathology (21 per cent). All cesarean sections were obstetrically indicated with the exception of 34 primary cesarean hysterectomies performed as definitive treatment of carcinoma in situ of the cervix. The operative procedures were compared in regard to the following characteristics or complications: operating time; incidence of blood transfusions, urinary tract injuries, postoperative bleeding, febrile morbidity, and other postoperative complications; and postoperative hospital days.

UNDER EMERGENCY circumstances, removal of the uterus at the time of cesarean section is accepted as a life-saving procedure. Elective removal of the puerperal uterus after an obstetrically indicated cesarean section simply for sterilization or for removal of a diseased uterus is controversial. The primary argument against elective cesarean hysterectomy is the reportedly high incidence of blood transfusions, urinary tract injuries, and postoperative morbidity.[1, 2]

The experience of the senior author has suggested that injury to the urinary tract is not a serious problem, that the incidence of blood transfusions can be reduced to acceptable levels if proper operative technique is used, and that in our institution postoperative morbidity after cesarean hysterectomy is less than after cesarean section.

The purpose of this study is to compare complications of elective total cesarean hysterectomy with those of elective cesarean section or cesarean section and tubal ligation during a 5 year period of time.

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Presented by invitation at the Eighty-sixth Annual Meeting of the American Association of Obstetricians and Gynecologists, Hot Springs, Virginia, September 4-6, 1975.

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Materials and methods

From January 1, 1970, through December 31, 1974, on the obstetric service at the University of Arkansas Medical Center, all patients undergoing an obstetrically indicated cesarean section who requested sterilization were offered the option of tubal ligation or hysterectomy. In addition, particularly during 1973 and 1974, primary cesarean section and hysterectomy were offered to patients who had uterine pathology for which a hysterectomy was indicated and had been scheduled 2 to 3 months postpartum. The primary indication in the latter group was carcinoma in situ of the cervix.

To evaluate the relative risk of cesarean hysterectomy and cesarean section with or without tubal

ligation, all abdominal deliveries from January 1, 1970, through December 31, 1974, were reviewed (Table I). During the study period there were 13,568 deliveries, of which 1,388 were abdominal deliveries on patients of more than 28 weeks' gestation (10 per cent cesarean section rate). Operations resulting in sterilization were performed on 1,047 patients (7.7 per cent). Fifty-four cesarean and cesarean tubal ligation records and one cesarean hysterectomy patient record were incomplete and not included in these data. There were no deaths or prolonged hospitalizations in the group that was not reviewed. Cesarean sections performed for vaginal bleeding were reviewed, classified as emergency, and excluded from the cesarean section study group; therefore, 806 elective cesarean section and 207 elective cesarean and tubal ligation patient records were studied.

A total of 257 cesarean hysterectomies were performed (18.5 per cent of abdominal deliveries) during the 5 year period of study. Twelve hysterectomies performed for bleeding were classified as emergency operations and excluded from this study as were two radical hysterectomies for invasive cervical cancer and one patient whose record was not available for study. Complications and results of 242 elective cesarean hysterectomies will, therefore, be compared with 806 elective cesarean sections and 207 elective cesarean sections with tubal ligation. The number of requests for surgical sterilization has increased dramatically in recent years. Due to limited operating time, postpartum tubal ligations are performed selectively. Thirty-five per cent of patients undergoing an elective cesarean section received a procedure that resulted in sterilization; 207 (17 per cent) tubal ligations and 242 (18 per cent) hysterectomies were performed. Fifty-four per cent of procedures resulting in sterilization in conjunction with cesarean section were hysterectomies.

Prenatal care and preoperative preparation. Approximately 50 per cent of our patients reside in the metropolitan area and the remainder are referred from throughout the state. During prenatal care, most patients received an interview concerning child spacing and family planning. If surgical sterilization was requested, a complete explanation was provided by the Public Health Nurse which included illustrations of the various procedures, i.e., postpartum tubal ligation or, if elective cesarean section was indicated, tubal ligation or hysterectomy. If uterine pathology existed which should be treated by hysterectomy, the patient was so counseled by the physician. During the last 3 years of the study, patients found to have a positive cervical cytologic smear either prior to or during pregnancy were evaluated by colposcopy. If the lesion was completely visualized and the examiner was confident that the cervix had been adequately evaluated and biopsied, diagnostic conization was not recommended. If indicated, cervical conization was performed during the second or early third trimester. Also, during the last 3 years, fetal maturity was estimated on all patients scheduled for elective cesarean section by performance of an outpatient amniocentesis and determination of a lecithin/sphingomyelin ratio. When fetal maturity was demonstrated, the elective operative procedure was scheduled accordingly. Appropriate sterilization papers were completed by the patient and her husband during the prenatal period.

Table I. Deliveries, 1970 to 1974

	Years					
	1970	*1971*	*1972*	*1973*	*1974*	*Total*
Total deliveries	2,826	2,851	2,746	2,511	2,634	13,568
Abdominal deliveries	252	299	264	294	279	1,388
Elective cesarean section	151	167	154	168	166	806
Emergency cesarean section	18	14	11	12	9	64
Cesarean tubal ligation	14	31	37	57	68	207
Postpartum tubal ligation	72	166	122	167	56	583
Elective cesarean hysterectomy	49	71	47	46	30	243
Emergency cesarean hysterectomy	0	3	3	4	2	12
Radical cesarean hysterectomy	0	1	1	0	0	2

After admission to the hospital, patients who were scheduled for a sterilization procedure, either in conjunction with cesarean section or by postpartum tubal ligation, were interviewed by the Chief Resident in Obstetrics. Institutional sterilization papers were completed and approved by a staff physician, who usually interviewed all patients who had elected hysterectomy for sterilization or definitive treatment of uterine pathology. Routine preoperative studies were completed and two to four units of whole blood were typed and cross-matched. Vaginal preparation was not routinely carried out.

Age. The age distribution of each group of patients is outlined in Table II. Approximately 15 per cent of patients who deliver on our service each year are age

Table II. Age distribution

Age	No. of patients			
	Cesarean hysterectomy	*Cesarean section*	*Cesarean tubal ligation*	*Total*
<16	0	95	2	97
16-20	21	421	43	485
21-25	70	198	78	346
26-30	68	53	35	156
31-35	41	22	25	88
36-40	29	16	13	58
41-45	13	1	10	24
>45	0	0	1	1
Total	242	806	207	1,255

Table III. Parity distribution

Parity	*Cesarean hysterectomy*	*Cesarean section*	*Cesarean tubal ligation*	*Total*
0	5	447	8	460
1	21	240	68	329
2	60	63	67	190
3	49	22	20	91
4	33	14	9	56
5	22	6	12	40
6 or >	52	14	23	89
Total	242	806	207	1,255

16 or less and one third are 18 or less, of which approximately 10 per cent are multiparas. Ninety-five elective cesarean sections were performed on patients less than 16 years of age; two patients, para one or two, received a medically indicated tubal ligation. The incidence of procedures resulting in sterilization increased from 13 per cent of those in the 16 to 20 year age group to 96 per cent of women over age 40. The incidence of hysterectomy increased from 20 per cent of elective cesarean sections in the 21 to 25 year age group to 51 per cent of patients over age 36.

Parity. Parity was defined as the number of deliveries prior to the pregnancy under consideration. The parity distribution of each group of patients is indicated in Table III. Eight para 0 patients received a medically indicated tubal ligation for the following reasons: heart disease, four; diabetes, three; hemoglobinopathy, one. Indications for hysterectomy performed on five para 0 patients were medically indicated sterilization (three), large degenerating uterine fibroids (one), and one patient was over 40 years of age and elected hysterectomy for sterilization.

A tubal ligation was performed on 21 per cent and hysterectomy on 6 per cent of para 1 patients. Among para 2 patients, 35 per cent received a tubal ligation and 32 per cent a hysterectomy. Parity of 3 or greater was recorded for 276 patients; 23 per cent of these patients received a tubal ligation and 57 per cent a hysterectomy, which was often performed for uterine pathology.

Operative procedure. Atropine was administered as a drying agent. Spinal or epidural anesthesia was used as the primary anesthetic in 85 per cent of elective cesarean sections and a local anesthetic was used in 2 per cent. General anesthesia was used as a primary anesthetic in 13 per cent and as a secondary anesthetic in 9 per cent of cesarean section patients. A low vertical abdominal incision was generally used, although a transverse incision was not contraindicated. If hysterectomy was to be performed, the bladder flap was completely developed prior to incising the uterus; the presence of adhesions often required sharp dissection which was performed more easily if the fetal head was in the lower uterine segment. The uterus was entered through a low cervical incision in 98 per cent of cesarean sections with or without tubal ligation; 73 per cent were transverse. Extension into the uterine fundus with elective cesarean section was reserved for obstetric complications such as malpresentation or an excessively large fetus. A low vertical incision was preferred with hysterectomy (95 per cent of operations), although in 22 per cent of patients the incision was extended into the fundus to facilitate delivery. A finger placed in the upper pole of a vertical incision by an assistant provided excellent traction on the uterus. Technical details of total cesarean hysterectomy have been published elsewhere.[3, 4]

All but eight of the tubal ligations were Pomeroy procedures, using plain catgut and excising a segment of Fallopian tube from each side for pathologic examination.

Nine patients undergoing cesarean section with or without tubal ligation received a unilateral oophorectomy for benign ovarian pathology and six underwent ovarian cystectomy. Twenty patients (8.3 per cent) undergoing hysterectomy received a salpingo-oophorectomy which was unilateral for bleeding (14) or a benign cystic teratoma (one) and bilateral in five women over age 36. Appendectomy was performed during 5 cesarean sections and 16 (6.6 per cent) hysterectomies. One bilateral hypogastric artery ligation was performed during cesarean section on a patient who was bleeding from Stage II cancer of the cervix.

Other operative procedures performed in conjunction with hysterectomy were removal of a wide vaginal cuff (34), lysis of adhesions (12), incisional or umbilical hernia repair (three), and Marshall-Marchetti-Krantz procedure (five). Adhesions were listed only if severe,

Table IV. Number of prior cesarean sections

Number of prior cesarean sections	*Cesarean hysterectomy*	*Cesarean section*	*Cesarean tubal*	*Total*	*Percent cesarean section (%)*
None	128	563	60	751	59.8
One	43	209	94	346	27.6
Two	48	30	46	124	9.9
Three	17	4	6	27	2.7
Four or >	6		1	7	
Total	242	806	207	1,255	100

Table V. Indications for primary cesarean section

Major indication	*Cesarean hysterectomy*	*Cesarean section*	*Cesarean tubal*
Fetopelvic disproportion	13	335	16
Malpresentation	23	114	21
Pre-eclampsia eclampsia	7	43	3
Prolapsed cord	2	22	3
Prolonged ruptured membrane	16	15	1
Diabetes	6	5	6
Isoimmunization	9	5	3
Uterine fibroids	5	1	0
Cancer cervix	34	4*	1*
Fetal distress	4	6	4
Vaginal bleeding	6	0	0
Miscellaneous	3	7	2
Total	128	557	60

*Invasive cancer of the cervix.

Table VI. Associated disease

Disease	*Cesarean hysterectomy*	*Cesarean section*	*Cesarean tubal*
Hypertension			
Without toxemia	13	4	10
With toxemia	5	5	4
Pre-eclampsia	9	134	11
Eclampsia	1	11	1
Chronic renal disease	1	4	2
Rheumatic heart disease	2	3	5
Diabetes	14	10	12
Epilepsy	3	8	2
Hemoglobinopathy	1	4	2

such as fixation of the uterus to the anterior abdominal wall. Dense adhesions were lysed during 31 cesarean sections and during 15 cesarean tubal ligation operations. During cesarean section two patients required a small bowel resection because of adhesions to a prior classical uterine scar.

Indications for cesarean section

A primary cesarean section was performed in 751 patients, which was 60 per cent of the 1,255 operations. Seventy per cent of cesarean sections, 29 per cent of cesarean tubal ligations, and 53 per cent of cesarean hysterectomies were primary cesarean sections. In 296 patients (23 per cent) an elective repeat and in 34 patients (carcinoma in situ of the cervix) an elective primary cesarean section was performed. Therefore, a scheduled operation on a nonlabor patient was performed with approximately 14 per cent of cesarean sections, 53 per cent of cesarean tubal ligation operations, and 45 per cent of cesarean hysterectomies.

Primary cesarean section in 751 patients was followed by tubal ligation in 60 (8 per cent) patients and hysterectomy in 128 (17 per cent) patients. A repeat cesarean section in 504 patients was associated with tubal ligation in 147 (29 per cent) and hysterectomy in 114 (23 per cent). A second cesarean section was performed in 346 patients; tubal ligation was performed on 94 (27 per cent) and hysterectomy on 43 (12 per cent) patients. The third, fourth, or fifth cesarean section on 158 patients was followed by a tubal ligation on 53 (34 per cent) and a hysterectomy on 71 (45 per cent) patients; 79 per cent of patients requested sterilization or uterine pathology warranted a hysterectomy. The number of prior cesarean sections in each group is recorded in Table IV.

Indications for primary cesarean section are listed in Table V. The principal indication was listed, although multiple indications were often present. Cesarean sections performed for diabetes, isoimmunization, or pre-eclampsia were usually preceded by a trial of induction of labor. Fetal distress or prolonged rupture of the fetal membranes often precipitated cesarean section; however, the primary disease process was listed as the indication for cesarean section. The principal associated diseases in each patient group are listed in Table VI. Vaginal bleeding from placenta previa (five) or abruptio placentae (one) was included in the hysterectomy group when the indication for hysterectomy was elective but was excluded from the other two groups. Fetopelvic disproportion and malpresentation were the most common indications for

Table VII. Indications for hysterectomy

Indication	Year					
	1970	*1971*	*1972*	*1973*	*1974*	*Total*
Elective sterilization	38	55	39	18	15	165
Medical sterilization	5	12	1	8	0	26
Uterine pathology	6	4	7	20	14	51
Carcinoma-in situ	2	1	5	17	9	34
Uterine fibroids	1	2	2	3	1	9
Amnionitis	3	1			4	8
Total	49	71	47	46	29	242

Table VIII. Preoperative and postoperative mean hematocrit values ± 1 S.D.

Patients	Cesarean hysterectomy	Cesarean section	Cesarean tubal
All patients	(N = 242)	(N = 806)	(N = 207)
Preoperative	35.6 ± 3.5	36 ± 4.3	35.2 ± 3.7
Postoperative	32.9 ± 4.2	32.9 ± 4.1	32.8 ± 3.9
Not transfused	(N = 195)	(N = 777)	(N = 197)
Preoperative	36 ± 3.2	35.8 ± 5.9	35.4 ± 3.6
Postoperative	32.5 ± 4.13	32.5 ± 5.7	32.4 ± 5.6
Transfused	(N = 47)	(N = 29)	(N = 10)
Preoperative	33.8 ± 4.24	32.14 ± 5.3	34 ± 4.4
Postoperative	31.0 ± 4.09	30.17 ± 3.2	29.4 ± 1.9

primary cesarean section. A breech presentation was the principal type of malpresentation. Fetal indications for cesarean section were: cord prolapse, Rh sensitization, diabetes, or evidence of a deteriorating fetal environment. Uterine fibroids was the indication for cesarean section in six patients and five underwent a hysterectomy. In four additional patients who had large fibroids a hysterectomy was not performed because they had requested conservative surgery to preserve a potential for fertility, they had not been counseled about hysterectomy, or the cervix had not been adequately evaluated prior to surgery.

Thirty-four patients requested primary cesarean hysterectomy as definitive treatment of carcinoma in situ of the cervix. In five additional patients the possibility of invasive cancer had not been excluded or in one instance an advanced cervical cancer was present; therefore, delivery was effected by cesarean section and a conization or definitive therapy was administered post partum. One hysterectomy specimen demonstrated microinvasive cancer.

Indications for cesarean hysterectomy

Indications for elective cesarean hysterectomy are listed in Table VII. Elective sterilization was the indication for 165 (68 per cent) operations; the incidence varied from 85 per cent in 1972 to 39 per cent in 1973. Medical indications were listed for 26 patients (11 per cent) but the incidence varied from year to year. Fifty uteri (21 per cent) were removed for the following pathologic conditions: carcinoma in situ of the cervix, 34; uterine fibroids, nine; and intrauterine infection, eight. Uterine pathology was the indication for 34 (45 per cent) hysterectomies during 1973 and 1974; 26 uteri were removed as definitive treatment of carcinoma in situ of the cervix. Patients found to have a positive cervical cytologic smear during pregnancy are referred to our institution for colposcopy from throughout the state. If the colposcopist was confident that the lesion had been adequately evaluated and hysterectomy was to be the definitive treatment, primary cesarean hysterectomy at term was discussed with the patient. Fetal maturity was judged primarily by amniocentesis and determination of the lecithin/sphingomyelin ratio. There has been one newborn loss in this group due to a congenital anomaly.

In nine patients large uterine fibroids, which in five instances interfered with labor and delivery, were treated definitively at the time of cesarean section.

Severe amnionitis, particularly in diabetic patients, remains a valid indication for cesarean hysterectomy and was the indication in eight patients.

The following medical indications for elective sterilization by hysterectomy were listed: isoimmunization, eight; diabetes, seven; hypertension, five; and miscellaneous, six.

Approximately 18 per cent of cesarean tubal ligations were medically indicated. The major indications were hypertension (14), diabetes (12) and rheumatic heart disease (five).

Operative complications

The principal operative complications of cesarean section with or without tubal ligation were lysis of adhesions (46 patients) and bladder entry during repeat cesarean section (four patients). Each bladder injury was recognized and repaired and healed satisfactorily.

Operative complications of cesarean hysterectomy were bladder entry (four), ureteral injury (one), bleeding adnexa (14), and operative hemorrhage. Three recognized and repaired bladder entries during repeat cesarean section healed uneventfully. One

unrecognized bladder injury during a repeat cesarean section and hysterectomy resulted in a vesicovaginal fistula that was repaired successfully at a second operation.

Partial severance of a ureter occurred during 1971 associated with bleeding from the uterine vessels. The injury was recognized and repaired and the patient was discharged on the eighth postoperative day; an intravenous pyelogram performed 3 months later was reported as normal.

Bleeding from an ovarian pedicle necessitated unilateral salpingo-oophorectomy in 14 patients; the bleeding was not considered to be of hemorrhage proportions.

The incidence of operative hemorrhage was difficult to evaluate. Although 30 patients received one or more blood transfusions preoperatively or during surgery, 17 patients were transfused postoperatively, usually in the recovery room or in association with postoperative bleeding. Eight patients received a cesarean section for vaginal bleeding and 13 patients were admitted with a hematocrit of less than 30 per cent. Significant hemorrhage usually occurred from the uterine veins or bladder flap.

Table IX. Blood transfusions

Amount of blood (c.c.)	*Cesarean hysterectomy*	*Cesarean section*	*Cesarean tubal*
0	195	777	197
500	10	10	4
1,000	22	16	4
1,500	9	1	1
2,000	3	1	1
2,500		1	
>2,500	3		
Total	242	806	207

Table X. Operating time

Operating time (min.)	*Cesarean hysterectomy*	*Cesarean section*	*Cesarean tubal*
<61	21	693	171
61-90	150	104	33
91-120	50	8	3
121-150	15	1	
151-180	3		
181 or >	3		
Total	242	806	207
Mean	86.74	48.6	51.1
±S.D.	30.32	19.1	14.5

Blood transfusions

The mean preoperative packed cell volume in each group ±1 S.D. was as follows: cesarean section, 36 ± 4.3 per cent; cesarean tubal ligation, 35.2 ± 3.7 per cent; cesarean hysterectomy, 35.6 ± 3.5 per cent. There was a less than 1 per cent difference between the groups. Transfused patients in each group had a significantly lower mean preoperative hematocrit than nontransfused patients in each group ($p < 0.05$). The last hematocrit value recorded prior to discharge from the hospital averaged approximately 33 per cent in each group as a whole; however, discharge hematocrit values on transfused patients averaged approximately 30 per cent. Additional postoperative transfusions were not administered unless clinically indicated. The preoperative and postoperative hematocrits for each group as a whole and those transfused or not transfused are summarized in Table VIII.

The quantity of blood administered to each patient in each group is summarized in Table IX. Transfusion was indicated with 29 (3.6 per cent) cesarean sections, 10 (4.8 per cent) cesarean tubal ligations, and 47 (19.4 per cent) hysterectomies. A preoperative hematocrit of less than 30 per cent was recorded for 28 cesarean sections (eight transfused) and eight cesarean tubal ligations (two transfused). One patient undergoing cesarean section bled profusely from a Stage II cancer of the cervix and required bilateral hypogastric artery ligation and transfusion of 2,000 ml. of blood.

Thirteen patients undergoing cesarean hysterectomy were admitted with a hematocrit of 30 per cent or less and were transfused. Indications for cesarean section in 47 transfused patients were previous cesarean section (23), uterine pathology, either fibroids (two) or carcinoma in situ of the cervix (five), cephalopelvic disproportion or malpresentation (11), prolonged rupture of fetal membranes (three), preeclampsia (two), and bleeding (one). Ten patients were considered to have had significant postoperative bleeding. Seventeen patients were transfused postoperatively. The incidence of transfusions varied from 33 per cent in 1970, the first year of the study, to 7 per cent in 1971. During the last 3 years the incidence has been 21 per cent.

Twenty-four of 86 patients who were transfused received one unit of whole blood, which probably accounts for the mean discharge hematocrit value of approximately 30 per cent among transfused patients. Our policy has been to transfuse to maintain stability of vital signs and not necessarily to replace blood loss completely. In recent years there has been a tendency to maintain an effective circulating volume in the operating room with intravenous fluids and a larger

Table XI. Postoperative complications

Complication	No. of patients					
	Cesarean hysterectomy		Cesarean section		Cesarean tubal	
	No.	%	No.	%	No.	%
Febrile morbidity	75	31	441	55	70	34
Urinary tract infection	31		160		33	
Pulmonary	7		19		4	
Wound	6*		25†		6	
Pelvic infection (total)	37		354		42	
Cuff infection		37				
Endometritis				354		42
Parametritis				40		
Pelvic abscess		2		2		
Suppurative phlebitis		2		3		1
Thrombophlebitis	1					
Postoperative						
Bleeding (total)	10					
Cuff hematoma		4				
Incisional hematoma		1				
Intra-abdominal		5				1

*Second-degree closure—2.
†Second-degree closure—10.

Table XII. Antibiotics administered

	No. of patients		
	Cesarean hysterectomy	Cesarean section	Cesarean tubal
Antibiotics administered	135(56%)	561(70%)	106(51%)
Penicillin	14	75	13
Streptomycin	6	59	10
Ampicillin	58	349	49
Cephaloridin	44	174	28
Tetracycline	40	138	18
Kanamycin	12	59	10
Cleocin	1	10	2
Gentamycin	1	8	1
Miscellaneous*	7	59	14

*Antibiotics administered specifically for treatment of urinary tract infection.

Table XIII. Postoperative hospital days

Days	Cesarean hysterectomy	Cesarean section	Cesarean tubal
< 5	1	17	6
5	20	121	58
6	96	323	86
7	74	205	33
8-10	30	105	19
11-14	13	29	3
> 14	8	6	2
Total	242	806	207
Mean	7.45	6.8	6.2
± 1 S.D.	3.83	3.8	1.7

number of transfusions are given in the obstetric recovery room.

Three patients undergoing hysterectomy received more than 2,500 ml. of whole blood. Two patients were transfused postoperatively and required secondary surgery for intra-abdominal bleeding and one sustained a ureteral injury and the operative procedure was prolonged.

Operating time

Operating time was the elapsed time from incision of the skin to completion of closure. The mean and distribution of operating times for each procedure are listed in Table X. Tubal ligation added less than 5 minutes to the average cesarean section, whereas hysterectomy added approximately 38 minutes. Twelve cesarean sections, three with tubal ligation, lasted longer than 90 minutes. Seven were primary and five were repeat operations. Only one of these patients required a transfusion. The operations were complicated by adhesions, repair of a bladder laceration or, in two instances, a small bowel resection.

The duration of six cesarean hysterectomies was over 150 minutes; three operations were performed in 1970 and three in 1971. Two operations were performed for fibroids that obstructed labor and each patient received a 1,000 ml. transfusion. Three operations were elective repeat cesarean sections; one patient weighed 280 pounds, another had multiple adhesions

and a large ventral hernia that was repaired, and the third had a preoperative hematocrit of 26 per cent and a dead hydropic fetus. The sixth patient sustained hemorrhage from the uterine vessels, requiring a 3,000 ml. transfusion, and a ureter was partially severed and repaired.

From 1972 through 1974 the mean operating time for 122 cesarean hysterectomies was reduced to 75 ± 20.7 minutes. Only 20 (16 per cent) operations took over 90 minutes to complete and four (3 per cent) took 121 to 150 minutes.

Postoperative complications

Postoperative complications experienced before discharge from the hospital were reviewed. Febrile morbidity, defined as a temperature elevation to 100.4° F. on 2 consecutive days after the first postoperative day, was recorded after 75 (31 per cent) cesarean hysterectomies, 441 (55 per cent) cesarean sections, and 70 (34 per cent) cesarean tubal ligations (Table XI). Electively scheduled operations on nonlabor patients correlated with the lowest incidence of postoperative febrile morbidity.

Antibiotics were administered after 135 (56 per cent) hysterectomies, 561 (70 per cent) cesarean sections, and 106 (49 per cent) cesarean tubal ligations (Table XII). Several antibiotics, seven in one badly infected patient, were administered to some patients. Antimicrobial agents administered specifically for treatment of urinary tract infection were included in a miscellaneous category and included methenamine mandelate, phenazophyridine hydrochloride, sulfisoxazole, and nitrofuratoin. Antibiotics were administered preoperatively only if there was an identified or strongly suspected infection present. Examples were a positive serologic test for syphilis, pyelonephritis, or prolonged rupture of the fetal membranes with an elevated temperature.

Urinary tract infection was recorded after 13 per cent (hysterectomy group) to 20 per cent (cesarean sections) of operations. Pulmonary complications were primarily atelectasis with or without pneumonia.

Urinary tract infections (16 per cent) and endometritis (20 per cent) were the primary causes of febrile morbidity after cesarean tubal ligation operations. One patient responded to treatment with heparin for suspected suppurative pelvic thrombophlebitis.

Wound infections were recorded after six hysterectomies, 25 cesarean sections, and six cesarean tubal ligations. Twelve secondary wound closures were performed, two after hysterectomy and 10 after cesarean section.

Endometritis was recorded after 354 (44 per cent) cesarean sections and in 40 patients there was an associated parametritis. In two patients the infection progressed to pelvic peritonitis and pelvic and ovarian abscesses; a hysterectomy and bilateral salpingo-oophorectomy were performed on the fourth and eleventh postoperative days, respectively. In the latter patient an inferior vena cava ligation was also performed and subsequent operations were performed for drainage of a subdiaphragmatic abscess and closure of an enterocutaneous fistula. Two patients did not respond to antibiotic therapy but did respond to heparin administration for suspected suppurative pelvic thrombophlebitis.

Vaginal cuff cellulitis or abscess was the cause of febrile morbidity after 37 (15 per cent) hysterectomies. Two patients were considered to have suppurative pelvic thrombophlebitis associated with ovarian and pelvic abscesses. Each required secondary surgery consisting of bilateral salpingo-oophorectomy and inferior vena cava ligation.

Postoperative bleeding requiring drainage or secondary surgery was not recorded after cesarean section but was noted after one cesarean tubal ligation and 10 cesarean hysterectomies. One patient, during cesarean tubal ligation, was found to have bilateral cystic ovarian enlargement from hyperreactio luteinalis, and a unilateral oophorectomy was performed to confirm the diagnosis. Intra-abdominal bleeding from the infundibulopelvic ligament necessitated abdominal re-entry on the day of surgery and religation of the ovarian vessels.

Ten patients were considered to have had significant bleeding after hysterectomy. The primary sites of bleeding were as follows: cuff hematoma, four; wound and cuff hematoma, one; and intra-abdominal bleeding requiring secondary surgery, five. Three vaginal cuff hematomas were drained vaginally and one extraperitoneally; vaginal cuff bleeders were also ligated on two of these patients. In one patient, on the third postoperative day, a 75 c.c. vaginal cuff hematoma was drained vaginally after which an extraperitoneal hematoma of similar size in a transverse incision was drained; inspection of the peritoneal cavity revealed no evidence of bleeding and the patient was discharged on the tenth postoperative day. In five patients there was intraperitoneal bleeding treated by bilateral hypogastric artery and specific vessel ligation and in one instance unilateral salpingo-oophorectomy. Two patients were operated upon within 2 to 4 hours postoperatively and three were re-entered on the fourth postoperative day. One patient received a third

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operation for a pelvic and ovarian abscess and suppurative pelvic thrombophlebitis (recorded with pelvic infections). Bleeding in one patient was caused by disruption of vaginal cuff sutures during vesical neck incision and vaginal packing immediately after hysterectomy; the patient was a paraplegic and had an ileal conduit.

Postoperative hospital days

The day after surgery was considered to be the first postoperative day and the day of discharge was the last day. The mean postoperative stay for each group is listed in Table XIII. Cesarean tubal ligation patients experienced the shortest stay, which correlated with a lower morbidity and a larger number of electively scheduled operations. The hysterectomy patients stayed an average of 0.65 days longer than the cesarean section patients and 1.25 days longer than cesarean tubal ligation patients.

Hospitalization was prolonged beyond 14 days in eight patients who underwent cesarean section without hysterectomy. One postoperative cesarean tubal ligation patient was treated for endometritis and hospitalized for 17 days, and a second was treated with heparin for suspected suppurative pelvic thrombophlebitis. Six prolonged hospitalizations which occurred after cesarean section were caused by the following complications: wound infection, two; endometritis, two; and infection requiring postpartum hysterectomy, two.

Eight patients remained in the hospital over 14 days after hysterectomy. Four patients required secondary surgery, one for postoperative bleeding, two for ovarian and pelvic abscesses, and one for secondary wound closure. Three patients developed a cuff hematoma and abscess. One patient was treated with heparin for suspected suppurative pelvic thrombophlebitis.

Late postoperative complications

Postoperative cesarean section or cesarean tubal ligation patients are referred to a Public Health Clinic for examination by a resident physician 6 weeks after discharge from the hospital. Cesarean hysterectomy patients are appointed to the departmental gynecology clinic, examined and referred to a Public Health Clinic. Patients exhibiting complications that warrant follow-up are maintained in or are referred back to the departmental gynecology clinic.

There was no record of readmissions to the hospital for complications related to cesarean section with or without tubal ligation. Several small incisional abscesses were treated on an outpatient basis. Four patients were treated for cervical cancer and one refused therapy. Ten patients underwent a hysterectomy, two of which were Schauta operations.

There were six recorded significant complications among hysterectomy patients after discharge from the hospital. Three patients required ligation of a bleeding vaginal cuff vessel 14 to 21 days postoperatively and one was readmitted to the hospital. One patient was readmitted on the twenty-first postoperative day for treatment of a vaginal cuff abscess. Another patient received a bilateral salpingo-oophorectomy ten months postoperatively for an ovarian abscess.

One vesicovaginal fistula occurred after elective repeat cesarean section and hysterectomy. A bladder injury was unrecognized and the fistula was confirmed on the fifth postoperative day by instillation of methylene blue into the bladder. The fistula was in the midline and small but did not heal spontaneously with catheter drainage. A successful repair was accomplished 2 months postoperatively.

Comment

The place of elective cesarean hysterectomy on our obstetric service has been reviewed by evaluating all elective abdominal deliveries performed after 28 weeks of gestation during a 5 year period of time. Cesarean sections and hysterectomies performed for hemorrhage were excluded to allow evaluation of complications and the need for blood replacement associated with elective operations in our patient population.

The incidence of cesarean sections during the 5 years was 10 per cent, of which 60 per cent were primary procedures. Thirty-four primary cesarean sections were performed to allow hysterectomy as definitive treatment of carcinoma in situ of the cervix; all other operations were obstetrically indicated.

Indications for elective hysterectomy were categorized as elective sterilization, medical sterilization or uterine pathology. Elective sterilization was the indication for 68 per cent of hysterectomies in the entire series, but only 44 per cent during the last 2 years of the study. Isoimmunization, diabetes, and hypertension were the major reasons for medically indicated sterilization.

During the last 2 years of the study, uterine pathology was the indication for 45 per cent of hysterectomies. Primary cesarean hysterectomy for definitive treatment of carcinoma in situ of the cervix has proved to be a very useful procedure. These patients are referred from throughout the state and encounter many problems related to expense, transportation, and care of children during a second hospitalization for elective hysterectomy 3 months

postpartum. Removal of an adequate vaginal cuff can be accomplished without undue difficulty by compressing the upper vagina between the thumb and forefinger. Cesarean hysterectomy should be considered for any multiparous patient undergoing cesarean section after prolonged rupture of the fetal membranes. This is particularly true if there is evidence of intrauterine infection.

Operative complications during cesarean hysterectomy were urinary tract injury, bleeding from an ovarian pedicle, and hemorrhage. All bladder lacerations that were recognized and repaired healed uneventfully after 10 days of catheter drainage of the bladder. One unrecognized injury was confirmed on the fifth postoperative day and required secondary surgery. An occasional vesicovaginal fistula is probably an unavoidable complication. One ureteral injury in 1971 was repaired primarily and healed without difficulty.

During approximately 5 to 10 per cent of cesarean hysterectomies, a unilateral oophorectomy is performed for bleeding from the ovarian pedicle.[5] The utero-ovarian pedicle is quite large and friable and if the ligature is tied too tightly or too close to the ovary it may tend to cut through the friable ovarian tissues.

Excessive blood loss is perhaps the major potential complication of cesarean hysterectomy.[3, 4] From these data one can expect an incidence of blood transfusion of approximately 20 per cent for elective cesarean hysterectomy and 4 per cent with cesarean section, with or without tubal ligation. One might speculate that the incidence of blood transfusion could be reduced by staff surgeons operating on a group of private patients.

Tubal ligation added less than 5 minutes to the operating time of the average cesarean section. Total hysterectomy increased the operating time by 38 minutes to a mean of 86.7 minutes. During the last 2 years the mean operating time for cesarean hysterectomy was reduced to 75 ± 20.7 minutes or 26 minutes longer than cesarean section.

Postoperative febrile morbidity according to the standard definition occurred after 55 per cent of cesarean sections, 34 per cent of cesarean tubal ligations, and 31 per cent of cesarean hysterectomies. The incidence of febrile morbidity was increased in patients who had been in labor, particularly if the membranes had been ruptured.

Postoperative bleeding was not a complication after cesarean section with the exception of bleeding from the infundibulopelvic ligament in one patient who had had a unilateral oophorectomy. One can expect a 4 per cent incidence of postoperative bleeding and 2 per cent incidence of abdominal re-entry after elective cesarean hysterectomy. A similar incidence was recorded in a previously published series of cases.[5]

The average postoperative stay after hysterectomy was 0.65 days longer than after cesarean section. Cesarean tubal ligation patients had the highest percentage of planned operative procedures before the onset of labor and the shortest postoperative stay.

Only serious late postoperative complications were brought to our attention. There was no record of late complications after cesarean section with or without tubal ligation that required readmission to the hospital. As one might expect after hysterectomy, three patients developed vaginal cuff bleeding 14 to 21 days postoperatively and one patient required a second operation to remove an ovarian abscess.

The emotional impact of hysterectomy on young women has not been considered in this discussion. A patient undergoing hysterectomy for the treatment of uterine pathology, by and large, can accept the procedure without undue concern. Removal of the uterus simply for sterilization, on the other hand, requires thorough preoperative preparation, which on our service is performed primarily by nonphysicians prior to the patient's admission to the hospital. After hospitalization the Chief Resident in Obstetrics and a staff physician again interview the patient and review the operation and possible complications.

The purpose of this report was not to discuss the pro and con of total cesarean hysterectomy vs. cesarean and tubal ligation but rather to record our experience with elective abdominal deliveries during a 5 year period of time. Each surgeon, on the basis of his own surgical experience and these and other data, must decide in conjunction with each patient the best method of sterilization or treatment of uterine pathology at the time of elective cesarean section.

We gratefully acknowledge the assistance of Dr. James Meade and Elizabeth Epley of the Division of Biometry at the University of Arkansas for Medical Sciences in the compilation and statistical analysis of these data.

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Discussion

DR. KEITH P. RUSSELL, Los Angeles, California. A discussant presented with Dr. Barclay's comprehensive and excellent review of his experience with over 250 cesarean-hysterectomies in the past 5 years soon develops two major areas for evaluation. One obvious consideration is the use of hysterectomy as a primary and elective method of sterilization. The other is in reference to the techniques employed in performing hysterectomy at the time of cesarean section, the technical aspects of which are critical in the evaluation of the procedure.

As regards the use of hysterectomy as a method of sterilization, this matter has been discussed and debated by most medical organizations repeatedly in the past several years. General facts regarding the procedure have been affirmed and reaffirmed and the usual reservations expressed. The cliches are well known: "Why use a sledge hammer to crack a walnut," and "Don't employ an elephant gun to kill a tsetse fly." The medical facts are also well known: a failure rate for tubal ligation of from 0.5 to 2.0 per cent, a subsequent incidence of gynecologic surgery in these patients of 25 to 35 per cent, a subsequent hysterectomy rate from 6 to 18 per cent, and a 1 to 2 per cent incidence of carcinoma of the cervix. In general, all reports support an increased incidence of gynecologic complications (including a need for hysterectomy) in patients who have had previous tubal ligations. If the listener gathers that this discussant favors the general concept of hysterectomy as an acceptable method of elective sterilization, he is quite right. I strongly support Dr. Barclay's utilization of this procedure as being in the best medical interests of the properly selected and evaluated patient.

The technical aspects of the performance of hysterectomy at the time of cesarean section present the more difficult consideration point for acceptance. The most significant problem in this regard has to do with the documented increased blood loss. There was a fourfold increase in the need for blood transfusion in the author's cesarean-hysterectomy group. With the realities of diminishing availability of whole blood in many institutions, the justified concerns over the incidence of hepatitis and other ramifications, this is an understandable deterrent to the procedure. Dr. Barclay in this paper does not address himself to the possible use of blood substitutes rather than whole blood transfusions to deal with this problem, but I would suggest that there are many instances in which such substitutes, or blood components with a lower potential for hepatitis, can and should whenever possible be utilized. Especially would it appear that in instances of one-unit transfusions other components might well serve the purpose, with greater utilization of washed packed red cells, even though these still carry some hepatitis threat. Except for this one area, that is the blood loss feature, the other complications such as febrile reactions, genitourinary injuries, and vaginal cuff infections exhibit comparable incidences in cesarean alone, cesarean plus tubal ligation, and cesarean-hysterectomy. In fact, Dr. Barclay has again documented that cesarean-hysterectomy has the lowest postoperative febrile morbidity of the three procedures. Those who have had extensive experience with cesarean-hysterectomy have repeatedly noted that, in general, patients following cesarean-hysterectomy do exceedingly well when not compromised by the problem of excessive blood loss at the time of the procedure.

Dr. Barclay's comprehensive report again substantiates the observation that cesarean-hysterectomy is not for the occasional operator. As with most other surgical procedures, a broad experience with the technique will lead to a greatly reduced incidence of complications. This has been also exhibited by Ballard in our area in his utilization of vaginal hysterectomy for combined therapeutic abortion and sterilization. When that procedure is limited to patients with less than 15 weeks' gestation, the morbidity has been surprisingly low when the technique is carried out or supervised by the experienced operator.

In conclusion, I would submit two questions to the author. Is not the use of antibiotics prophylactically even more justified with this procedure? Have there been variations in the technique as regards the open vs. closed vaginal cuff management with or without drains?

DR. BARCLAY (Closing). The suggestion that blood substitutes or washed red cells be used when a single unit transfusion is likely is a good one. We have not used prophylactic antibiotics. One result of this study is our realization that primary cesarean section on patients who have been in labor is associated with postoperative febrile morbidity in at least 50 per cent of patients. We are in the process of setting up a prophylactic antibiotic study. We realize that studies have been done elsewhere but I think we should study our own patient population in our own environment and arrive at our own conclusions.

Operative technique is particularly important in

performing this operation. Although there is not time to go into any detail, I would like to emphasize the importance of sharp dissection, that the bladder flap is completely developed before the uterus is opened, and that all vascular pedicles including the uterine vessels are clamped before any are tied.

The final question was whether or not the vaginal cuff is closed or drained. I personally close the vaginal cuff but do not use drains. I thought all of our residents were closing the vaginal cuff until I recently found that an occasional resident prefers to secure hemostasis with a continuous suture and leave the cuff open.

Cell kinetics and programmed chemotherapy for gynecologic cancer

I. Squamous-cell carcinoma

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The results of current investigation into cellular kinetics of both normal and abnormal human genital tissue have stimulated interest in programming chemotherapeutic drug regimens for the treatment of genital cancer. This report outlines the results of cell kinetic studies of normal and abnormal squamous epithelium of the female genital tract and presents preliminary results of a regimen utilizing methotrexate, hydroxyurea, and vincristine in patients with far-advanced squamous-cell cancer of the vulva, vagina, and cervix. A total of 92 patients have received 444 courses of programmed chemotherapy by either pelvic intra-arterial infusion or systemic administration of the drugs. Our results are presented and the theoretical basis of the research is discussed.

ALTHOUGH it is accepted that systemic chemotherapy is effective in metastatic trophoblastic disease, and at times is of value in the treatment of advanced carcinoma of the ovary, breast, and endometrium, it is generally agreed that chemotherapy has been ineffective in the treatment of squamous-cell carcinoma arising in the female genital tract.[1–3] However, a single report by Sullivan and co-workers[4] in 1960 indicated exceptional tumor regression in four patients following the continuous 24 hour administration of the antimetabolite, methotrexate, by way of arterial infusion of the pelvis. In one patient with Stage IV squamous-cell carcinoma of the cervix treated only by chemotherapy, complete tumor regression was obtained and the patient was apparently free of disease 37 months after treatment.[5] To our knowledge, this is the only reported case of a long-term total remission of Stage IV squamous-cell carcinoma of the cervix treated only by chemotherapy. Subsequently others have utilized regional infusion chemotherapy for squamous-cell carcinoma of the pelvis with varying degrees of success.[6, 7]

Our interest in programmed chemotherapy was stimulated by our studies of normal and abnormal cell proliferation kinetics in human genital tissues.[8] It was our feeling that chemotherapy utilizing various cytotoxic agents could be programmed on a scientific basis if knowledge of the cell generation cycle in normal and malignant human tissues was available. Therefore, we have used a technique of local tissue injection of minute amounts of thymidine-^{3}H to study

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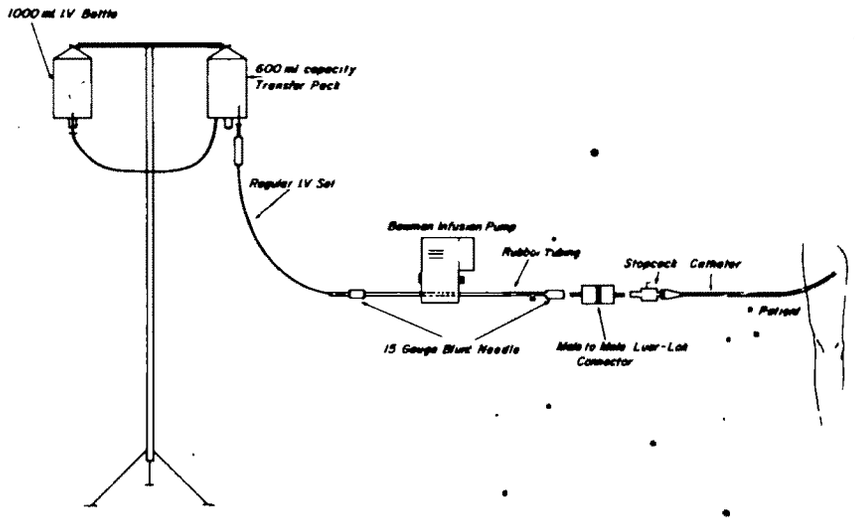


Fig. 1. Schematic representation of intra-aortic infusion of pelvis with percutaneous femoral catheterization and Bowman infusion pump.

normal and abnormal cell proliferation kinetics in human cervical, vaginal, and vulvar tissues in vivo. This report will summarize our experience with cell proliferation kinetics in human genital tissues as well as present preliminary results with programmed chemotherapy utilizing methotrexate (MTX), hydroxyurea (HU), and vincristine (VINC).

Materials and methods

Studies of cell proliferation kinetics are obtained on patients admitted to the Gynecologic Oncology Service at the University of Miami School of Medicine. Conditions such as dysplasia, in situ carcinoma, and invasive carcinoma of the cervix, vagina, and vulva have been studied. Candidates for programmed chemotherapy have cell kinetic studies both prior to and during various phases of chemotherapy.

Cells in human genital tissues that are actively synthesizing DNA are labeled by injecting 5 μc (in 0.1 ml.) of tritiated thymidine into marked sites in the tissues. Punch biopsy specimens (4 mm.) are obtained from normal or neoplastic cervix, vagina, or vulva at appropriate time intervals depending upon the information desired. Our methods for analysis of autoradiographs in the study of the generation cycle in normal human cervix and vagina have been published previously.[8] In studying cell kinetics in abnormal tissues, similar techniques are used with the exception that cell counts are made from enlarged photographs of autoradiographs to ensure that cell counts are accurately made.

All patients for pelvic infusion chemotherapy have a tissue diagnosis of persistent or recurrent squamous-cell carcinoma following complete treatment in the conventional manner with surgery and/or radiation therapy. Although it is not possible to completely exclude distant metastases, each patient treated is evaluated with intravenous pyelography, renal and liver function studies, and other radiographic techniques to exclude distant metastases prior to chemotherapy. No patient with findings consistent with tumor spread beyond the pelvis is included in the infusion group.

Patients undergoing programmed *intra-arterial infusion chemotherapy* have percutaneous placement of a catheter into the femoral artery by the Seldinger[9] technique. The catheter is secured to the skin with a silk suture after the tip has been placed just above the bifurcation of the aorta. The accuracy of the placement is checked by both arteriography and radioactive pelvic scan with macroaggregates of radioiodinated serum albumin. A schematic representation of the infusion setup is shown in Fig. 1. A constant infusion of parenteral fluid must be maintained, after the catheter is placed in the artery, with a Model 4 Bowman infusion pump or a blood pressure pump surrounding a plastic blood transfer pack filled with 5 per cent dextrose and water.

The chemotherapeutic program for patients with disease limited to the pelvis consists of three sequential steps: (1) MTX (50 mg.) in 600 ml. of 5 per cent dextrose and water is infused over a 24 hour period, thus delivering a high concentration of methotrexate to the pelvis. To prevent systemic toxicity of MTX that escapes into the circulation, folinic acid is administered intramuscularly in doses of 6 mg. every 6 hours during

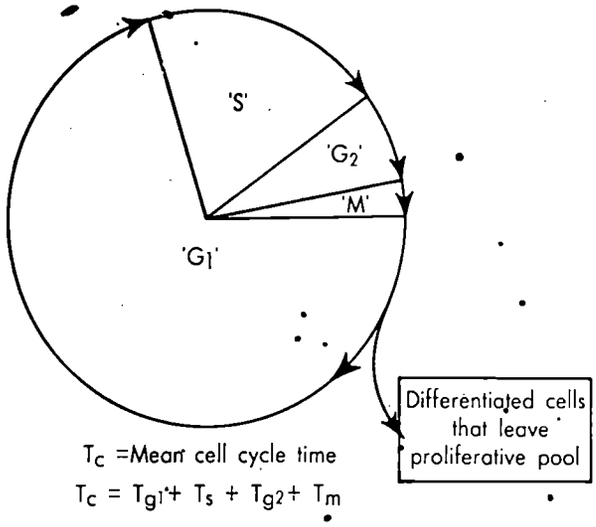


Fig. 2. Schematic representation of cell generation cycle and its component parts.

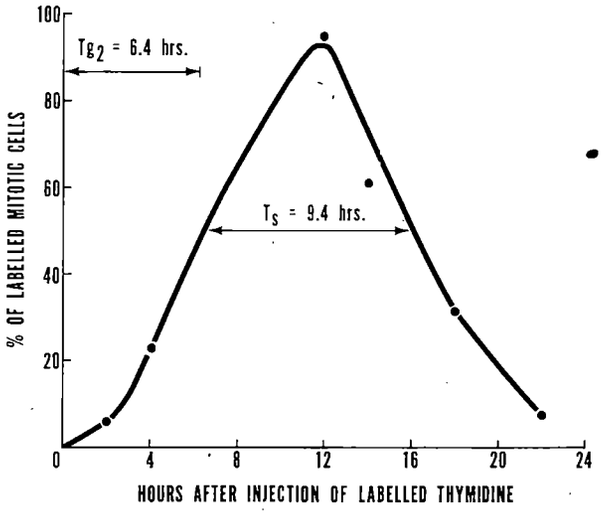


Fig. 4. DNA synthesis curve for squamous-cell carcinoma of vulva in situ.

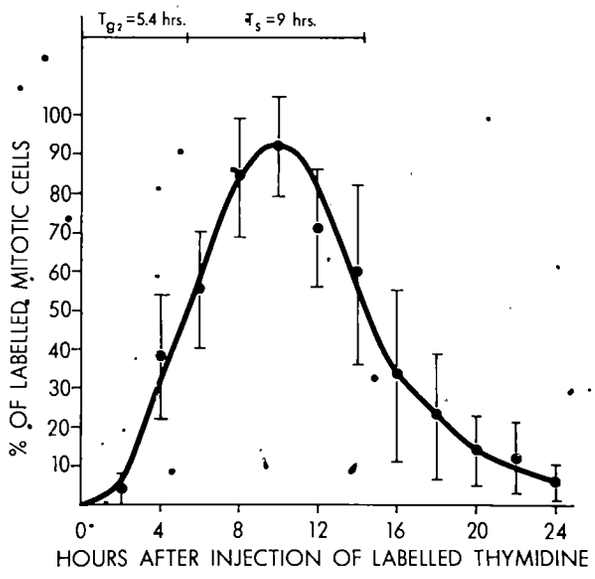


Fig. 3. Composite DNA synthesis curve of normal human cervical and vaginal epithelium based on mean values of per cent mitosis in 10 patients. Bars represent 95 per cent confidence levels.

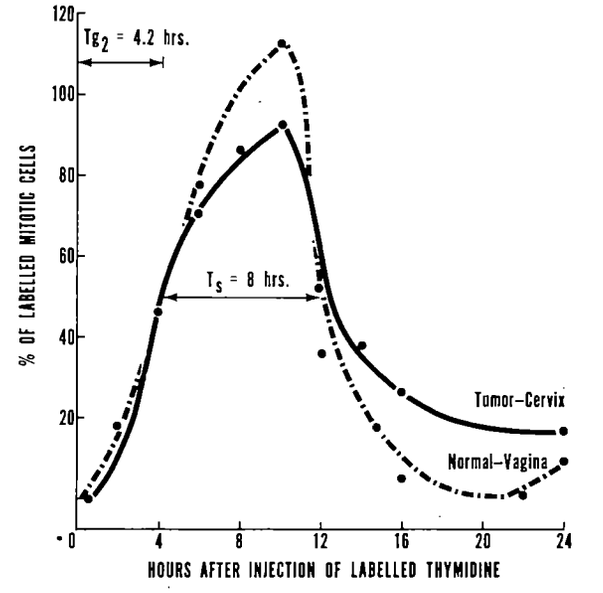


Fig. 5. DNA synthesis curves for invasive squamous-cell carcinoma of cervix and normal squamous epithelium of vagina in the same patient.

the treatment period. MTX is administered for 4 consecutive days, delivering a total dose of 200 mg. (2) One hour after completing the MTX therapy, HU (4.0 Gm.) is given by mouth as a single dose. (3) Eight hours after the MTX infusion is completed, VINC (2.0 mg.) is given by way of the femoral catheter and infused over a 1 to 2 hour period.

The triple therapy of methotrexate, hydroxyurea, and vincristine constitutes one course of therapy. The usual interval between courses of therapy is 48 hours, during which time the femoral catheter is maintained in place and 5 per cent dextrose is infused. The total number of chemotherapeutic infusions varies, depending upon the patient's ability to tolerate the procedure and/or the appearance of toxic manifestations of the drug program.

Patients selected for programmed *systemic chemotherapy* are patients with persistent, recurrent, or metastatic squamous-cell carcinoma following complete treatment in the conventional manner with

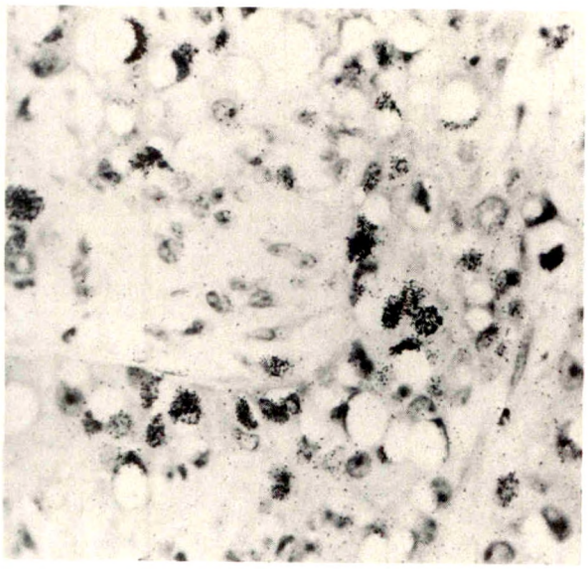


Fig. 6. Autoradiograph of squamous-cell carcinoma 20 minutes after local injection of thymidine-^{3}H. Labeling index was 27 per cent prior to infusion of MTX.

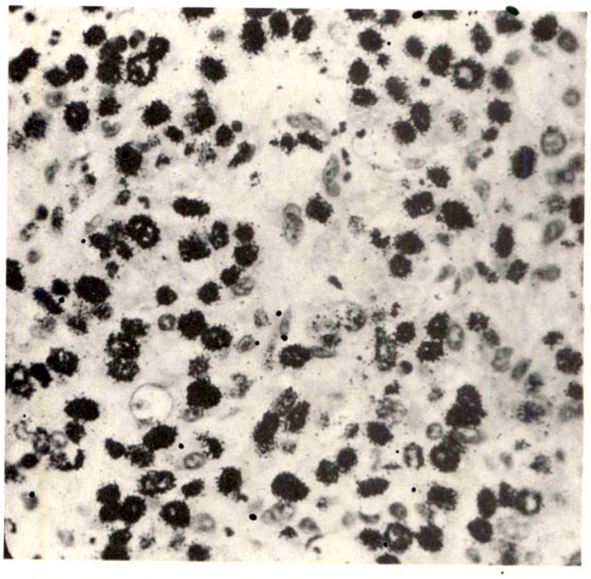


Fig. 7. Autoradiograph of squamous-cell carcinoma demonstrating marked increase in labeled cells (72 per cent) that were thymidine deficient after 96 hours of intra-aortic infusion of MTX.

surgery, radiation therapy, or both. Some of these patients have presented only with distant metastasis. Some have had local recurrence, but have been selected for systemic chemotherapy rather than infusion chemotherapy because of medical problems such as peripheral vascular disease. A third group of patients have both local recurrence and distant metastasis. All patients have had either biopsy-proved persistent or recurrent cancer or radiologic evidence of distant metastasis such as lung lesions or metastasis to bone. Methotrexate, hydroxyurea, and vincristine are given systemically by the following schedule.

1. Patients receive 15 mg. of MTX daily for 4 consecutive days followed by an intramuscular injection of 6 mg. of folinic acid on the morning of the fifth day. *The dosage of MTX must be reduced if there is impairment of renal or hepatic function.*

2. Following the folinic acid, 4 Gm. of HU are given by mouth during a 1 hour period.

3. Approximately 6 hours after HU, the patient receives an intravenous injection of 2 mg. of VINC. This course of therapy is administered every 3 weeks if the white blood count is above 3,000 and the platelet count above 100,000. If the hemogram is depressed it is repeated weekly until the critical values are reached, when another course of therapy is instituted. Periodic evaluation of liver and renal function is necessary so that the correct dose of MTX may be determined. It is mandatory that blood urea nitrogen (BUN) and creatinine levels be normal since MTX is excreted primarily by the kidney and severe toxicity will result if a full dose of MTX is given when renal function is poor.

Results

Cell kinetics. The four phases of the proliferative cell cycle are illustrated in Fig. 2. Following mitosis *(M)*, the daughter cells enter the postmitotic G_1 phase, where one of the cells may become a differentiated cell leaving the proliferative pool while the other cell may continue as a progenitor cell to reproduce again. In order for this cell to reproduce it duplicates its content of DNA during the DNA synthesis *(S)* phase. After DNA synthesis, there is a relatively short postsynthetic G_2 period before the next mitotic phase. A balance between dividing and nondividing cells has been defined in the normal human cervical and vaginal epithelium under varying degrees of estrogen stimulation.[8] In Fig. 3, a composite DNA synthesis curve for normal human vagina and cervix from 10 patients is plotted. From this curve, the S phase and G_2 phase can be measured as 9 and 5.4 hours, respectively. DNA synthesis curves are also plotted for in situ and invasive squamous-cell carcinoma (Figs. 4 and 5). Very little difference has been found in the S and G_2 phases between normal and neoplastic tissues.

In an effort to determine a mean cell generation cycle for squamous-cell neoplasia in the cervix and vagina, labeling indices have been tabulated for 50 patients. The mean labeling indices for patients with cervical dysplasia, carcinoma in situ, and invasive squamous-cell carcinoma are 16.6, 21.2, and 27.2

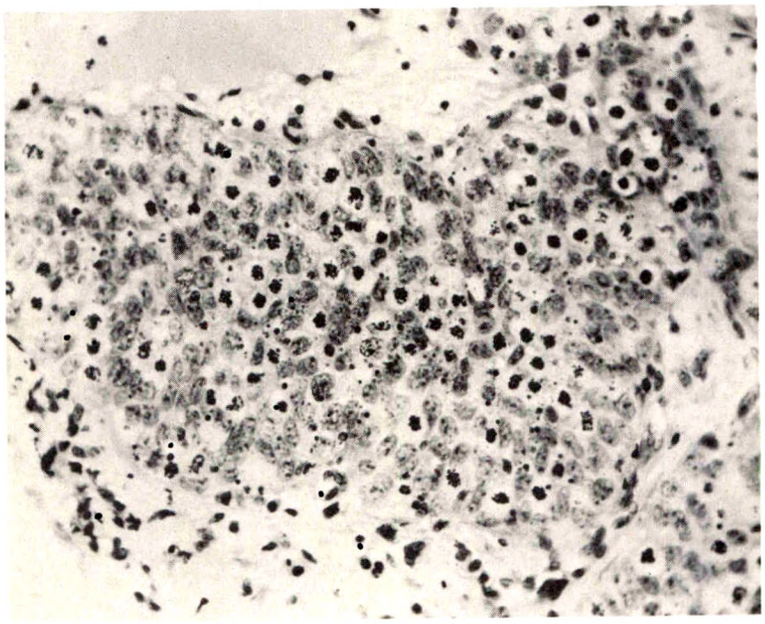


Fig. 8. Squamous-cell carcinoma in patient 24 hours after pelvic infusion of vincristine. Note numerous cells arrested in metaphase of mitosis (17 per cent).

Table I. Means of the labeling indices of squamous-cell neoplasia

Tissue	*No. of patients*	*Labeling index of whole proliferative pool (%)*
Dysplasia	9	16.6 ± (4.16)*
Cancer in situ	12	21.2 ± (2.46)
Invasive cancer	29	27.2 ± (2.78)

*Values in parentheses represent twice the S.E.

Table II. Germinative cell cycles in normal and neoplastic squamous tissues

Tissue	*L.I.* (%)*	T_s *(hr.)*	T_{g2} *(hr.)*	T_m *(hr.)*	T_{g1}	T_c *(hr.)*	T_c *(days)*
Normal	14.3	9	5.4	0.17	48	63	2.6
Cancer in situ	21.2	9	5.8	0.4	27.8	44	1.8
Invasive cancer	27.2	9	6.3	0.5	15.2	33	1.4

*L.I. = labelling index.

respectively (Table I). Using the determined values for S phase (in hours) and labeling indices, cell generation cycles for the various degrees of squamous neoplasia have been calculated (Table II). It will be noted that T_s, T_{g2}, and T_m vary only slightly when one compares normal to neoplastic tissues. However, there is progressive shortening of the entire generation cycle, T_c, when one compares normal tissue to dysplastic, and then to neoplastic, tissues. Shortening is primarily due to a decrease in the postmitotic G_1 phase.

Chemotherapy and cell cycle kinetics. Cytotoxic agents have been chosen for the treatment of invasive squamous-cell carcinoma based on their mode of action and the cellular kinetics of the tumor. The effects of methotrexate on squamous-cell carcinoma have been studied by obtaining labeling indices prior to infusion of MTX and after 96 hours of infusion. As seen in an autoradiograph of squamous-cell carcinoma prior to infusion of MTX, the labeling index before chemotherapy is 27 per cent (Fig. 6). However, after 96 hours of pelvic infusion chemotherapy with MTX, autoradiographs demonstrate marked uptake of tritiated thymidine (Fig. 7). The increase in labeling index to 72 per cent reflects the blockade of cells entering or already in the S phase that became thymidine deficient and are unable to complete DNA synthesis.[10, 11] Hydroxyurea was chosen as the second cyclic agent since its specific action appears in cells synthesizing DNA by inhibition of ribonucleotide reductase. Cells in the S phase are lethally damaged by HU, whereas cells in the G_1 phase are prevented from entering the S phase.[12]

The third agent, vincristine, was selected because of its action during either the G_2 or M period producing a metaphase arrest.[13] Since the tumor population would be substantially synchronized in S phase after a 96 hour infusion of methotrexate or, as in the case of systemic

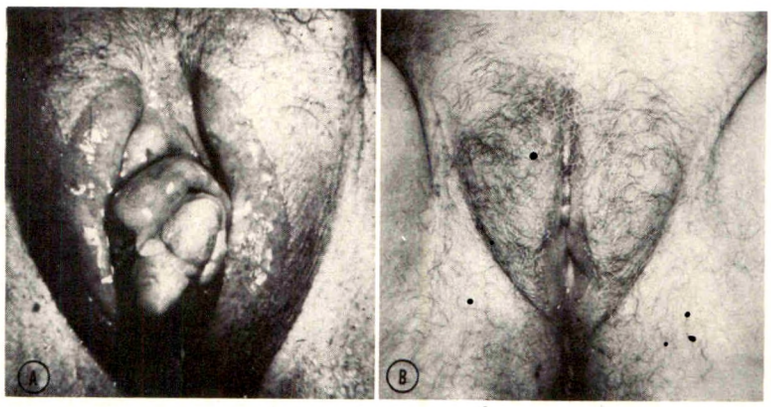


Fig. 9. Composite of patient with squamous-cell carcinoma of vagina with extension to vulva. *A*, Photograph taken before infusion chemotherapy; *B*, photograph taken 4 months after infusion chemotherapy.

therapy, by the 4 day course of oral methotrexate, it was then planned to manipulate the majority of these cells synchronously into the G_2 and M phases where vincristine should be very effective on a relatively high percentage of tumor cells. Vincristine-produced metaphase arrest in squamous-cell carcinomas is seen in Fig. 8.

Clinical applications of programmed chemotherapy

Patients with regional disease. Thirty-six patients with regional disease received 100 programmed infusions with relatively few complications. The first 12 patients received MTX only. The second group of nine patients received MTX and VINC. The third group consisted of 15 patients who received the full programmed regimen of MTX, HU, and VINC, as previously outlined. The average number of infusions per patient was 2.8, with a maximum of 11 and a minimum of 1. The average survival time of all patients receiving infusion chemotherapy was 12.8 months, with a maximum of 8 years and a minimum of less than 1 month. Patients were evaluated objectively and subjectively, with the most dramatic subjective response being pain relief. Twenty-two of 36 (61.1 per cent) patients have had significant or total relief of pain, usually after the first infusion. Patients receiving large doses of morphine every 2 to 3 hours usually required little or no analgesic 48 to 72 hours after the infusion was started. In six of 14 patients who were judged to have little or no relief of pain, a review of the arteriograms and radioactive pelvic scans demonstrated that little infusate had reached the pelvic tumor masses. These treatment failures occurred in patients who had been treated previously with radiotherapy and it is likely that radiation fibrosis or arterial obstruction secondary to the tumor masses prevented adequate infusion of cytotoxic agents to the tumor. Although initially several courses of therapy were given, it was later learned that if no response was noted during the first 5 day infusion, subsequent therapy was of little value. Therefore, if no response was obtained with the first infusion, chemotherapy was discontinued. Objective response of the tumor was defined as (1) greater than 50 per cent regression, (2) 50 per cent regression, (3) 25 per cent regression, (4) no change (i.e., neither progression nor regression), and (5) progression of tumor growth. Six patients had greater than 50 per cent regression, two patients had 50 per cent regression, one patient had 25 per cent regression, and four patients remained stable. Twenty-three patients had progression of disease despite chemotherapy. Of the six patients having greater than 50 per cent regression of tumor, two had total regression of tumor and are alive 8 and 7 years following completion of the infusion chemotherapy. Five patients died prior to discharge from the hospital subsequent to pelvic infusion. One died from congestive heart failure, two from sepsis, one from pulmonary embolization, and one died following amputation of an extremity secondary to peripheral embolization related to catheter placement. With the exception of the patient who suffered peripheral embolization, these deaths may or may not have been related to the infusion procedures. However, it is emphasized that all patients have had advanced, unresectable, squamous-cell cancer that has been treated completely in the conventional manner with surgery, radiotherapy, or both. Only three of the 36 patients are alive at this time, but some of the patients who died have had apparent

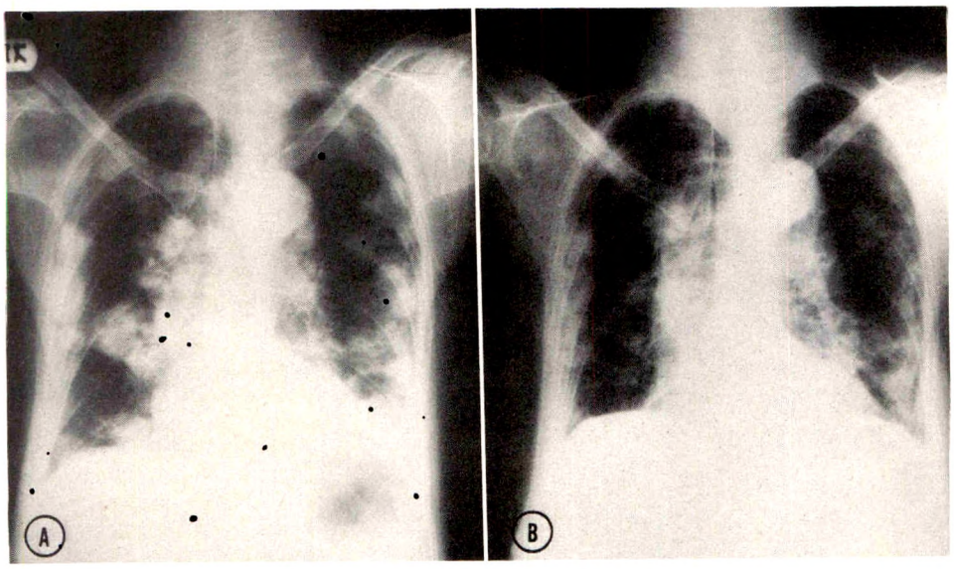


Fig. 10. Radiographs of chest demonstrating pleural effusion secondary to metastatic squamous-cell carcinoma *(A)* and disappearance of effusion after systemic programmed chemotherapy *(B)*.

complete control of pelvic disease, dying due to distant metastasis.

Of the two patients who have been long-term survivors, one was treated with radiotherapy for Stage III squamous-cell carcinoma of the cervix in July and August, 1965. Recurrence of carcinoma was detected when she presented with hydronephrosis and weight loss of 66 pounds. Exploratory celiotomy revealed that pelvic exenteration was impossible due to tumor fixation to the pelvic walls. In January, 1967, a 5 day infusion was followed by marked tumor regression and disappearance of pain. Five separate infusions were given and in August, 1967, intravenous pyelography demonstrated a normal collecting system without hydronephrosis. At the present time she is working, without complaints, has gained 70 pounds, and is clinically free of cancer. Another survivor was treated by radiotherapy for carcinoma of the vagina in May, 1967. Tumor recurrence with involvement of the vulva and fixation to the pubic rami was noted in October, 1967. Two infusions, each lasting 6 days, were given in October, 1967, and January, 1968. After the second infusion, the vulva appeared normal (Fig. 9). At present, the patient is without complaints except for excessive weight gain and vaginal stenosis.

Patients with disseminated disease. Thirty-four patients with systemic disease received 315 courses of programmed chemotherapy. An additional 22 patients received 29 courses of therapy, but were excluded from the study as they received less than three courses and therefore represent insufficient data for detailed analysis. Of these 22 patients, two are alive but have not completed three courses, three refused therapy prior to completing three courses, five patients were lost to follow-up, 10 patients died of their disease in less than 3 months, and two patients died secondary to toxicity of the chemotherapy. Of those patients who completed at least three courses of therapy, the average number of courses has been 9.1, with the maximum being 27 courses and the minimum being three courses. The average survival of these patients was 9.6 months, with a maximum of 26 months and a minimum of 3 months.

Observations were again subdivided into subjective and objective response. Subjectively, 13 patients were felt to have improved with total or partial relief of presenting symptomatology. Seven patients have experienced no significant improvement but have not deteriorated for at least 3 months and are considered to have remained stable. Fourteen patients had tumor progression and their condition was felt to have worsened despite the chemotherapy.

Objectively, six patients have had greater than 50 per cent regression of tumor masses, three patients have had 50 per cent regression, three patients have had 25 per cent regression, and seven patients have remained stable with neither progression nor regression over at least 3 months. Fifteen of 34 patients have experienced definite progression of disease. Five patients illustrated significant tumor response to systemic chemotherapy. Two patients with squamous-cell carcinoma were found to have lung metastasis with pleural effusions and were placed on the systemic regimen of MTX, HU, and VINC. Both patients had lesions that could be visualized radiographically. After two complete courses

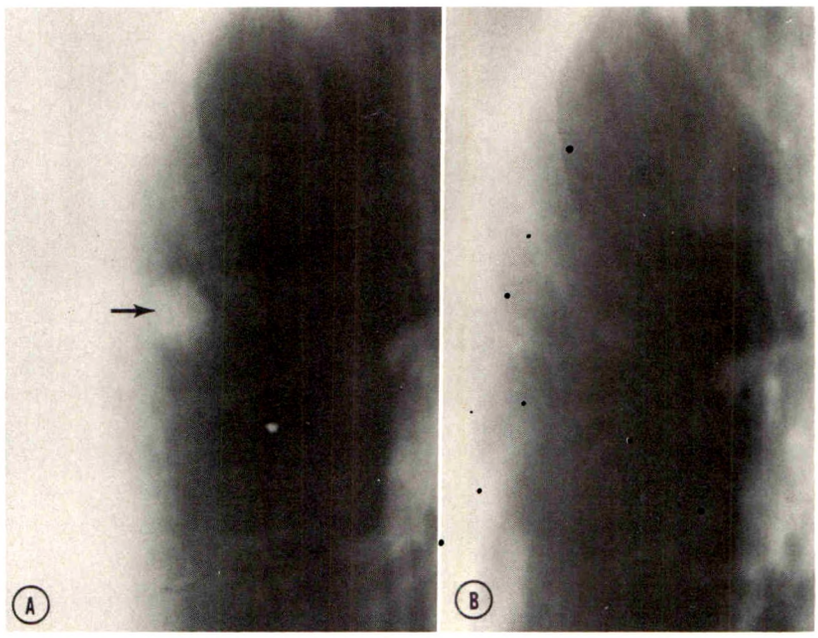


Fig. 11. Radiographs (tomograms) of patient with metastatic squamous-cell carcinoma *(A)* and disappearance of metastatic nodule after systemic programmed chemotherapy *(B)* (20 courses).

of therapy, the pleural effusions in both patients disappeared and significant decrease in the lung lesions was noted (Fig. 10). Both patients subsequently died due to disseminated tumor, one surviving for 8 months and the other for 14 months. A third patient was discovered to have recurrent carcinoma of the cervix metastatic to the lung and after seven courses of systemic chemotherapy, the previously noted lung lesion could no longer be identified. She received a total of 20 courses of systemic chemotherapy before she died of myocardial infarction. At autopsy a small focus of squamous-cell carcinoma was found in the lung. This area of carcinoma corresponded to the nodule seen earlier which had decreased to the point that it was no longer visible radiographically (Fig. 11). A fourth patient with lung metastasis has had complete disappearance of lesions after 17 courses of systemic chemotherapy. Ten weeks after discontinuing therapy a radiograph of the chest is normal. The fifth patient is included through the courtesy of Dr. Myron Lutz at the Medical University of South Carolina. This patient underwent radical vulvectomy for Stage III carcinoma of the vulva. Approximately 1 year after radical vulvectomy, local recurrence was noted, but she refused further treatment by surgery or radiation therapy. After a single course of systemic MTX, HU, and VINC, the tumor exhibited marked regression, as seen in Fig. 12. Unfortunately, severe bone marrow depression with sepsis resulted and subsequently she died. Of the 34 patients who received programmed systemic chemotherapy, 24 have died and 10 are alive at the present time. The longest survival to date is 26 months.

The over-all results for the 34 evaluable patients who received programmed systemic chemotherapy are shown in Fig. 13. The survival time for patients responding to therapy has been compared to the survival time for patients who failed to respond. The survival curve for the untreated group was obtained from hospital records of patients who died of recurrent squamous-cell carcinoma prior to our study of programmed chemotherapy. It is apparent that nonresponders have a survival time that is nearly identical to that of the untreated group whereas responders to chemotherapy have experienced longer survival and a longer interval free of tumor progression.

When delineating significant toxicity related to systemic chemotherapy, all patients have experienced hair loss, nausea, and stomatitis. Eight patients experienced vomiting or diarrhea requiring hospitalization. Three patients suffered sufficient depression of bone marrow to require admission and reverse isolation. One patient developed a peripheral neuropathy which probably was secondary to prolonged use of vincristine. One patient in the group who received at least three courses of therapy has died as a direct result of the chemotherapy due to overwhelming sepsis secondary to depression of the bone marrow. Two additional patients, mentioned previously, died as a result of the chemotherapy prior to completion of three courses.

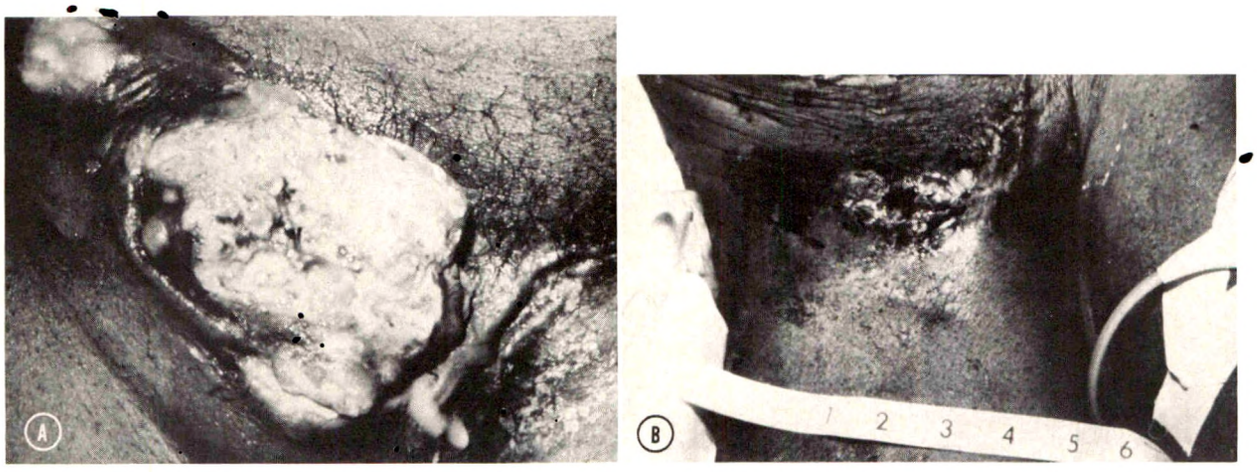


Fig. 12. Composite of patient with recurrent squamous-cell carcinoma of vulva *(A)* and marked regression of tumor after one course of systemic programmed chemotherapy *(B)*. (Photographs courtesy of Dr. Myron Lutz, Medical University of South Carolina.)

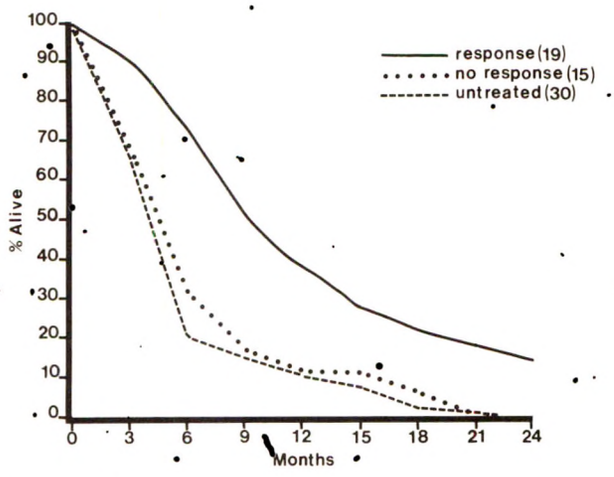


Fig. 13. Survival curve (actuarial survival) of responders to programmed chemotherapy compared to nonresponders and untreated patients.

Comment

The advantage of using cell proliferation kinetics of tumor tissue to program chemotherapy is to place chemotherapy on a more scientific basis than the empiricism of the past. Cell proliferation studies have been used to determine the life cycle of genital tumor cells so that the cycle could be pharmacologically manipulated by drugs like MTX, HU, and VINC. The principle of the triple-therapy schedule was developed to maximize the cytotoxic effects of chemotherapeutic agents on the tumor cell population by using cell synchronization techniques for both regional infusion and systemic chemotherapy. The initial infusion or oral administration of MTX attempts to synchronize most of the tumor cells into the DNA synthesis (S) phase. While these cells are trapped in the S phase, they are exposed to HU, which is also an S-phase–specific agent, and can preferentially destroy a larger portion of the tumor cell population. The third part of the schedule is to push the surviving cells out of the S phase synchronously with folinic acid and into another susceptible phase (G_2 and/or M phase) where VINC is cell-cycle specific. Regional infusion with programmed chemotherapy permits a maximum concentration of drugs to reach the tumor mass without significant systemic toxicity. Where metastatic disease is present beyond the pelvis, it is reasonable to assume that the kinetic activity of the distant tumor cell behaves in a similar manner to the primary tumor and would respond to chemotherapeutic scheduling. Systemic programmed chemotherapy has the advantage of prolonged outpatient usage; however, potential systemic toxicity becomes a greater problem and often doses must be adjusted.

Several aspects of the material presented here should be emphasized. The data regarding cell kinetics of human squamous-cell genital carcinoma are preliminary. Factors considered important clinically, such as histologic cell type (i.e., large vs. small, keratinizing vs. nonkeratinizing, mature vs. immature), have not been investigated with respect to possible kinetic differences. The cell generation cycle has been defined only as an average estimate since it is possible that in an asynchronous cell population there will be considerable

variation from one cell to another. Theoretically, if each tumor cell had the same generation cycle, one course of chemotherapy covering an entire cell cycle while utilizing the right cytotoxic agents sequentially should eliminate the tumor. Although some tumors regressed rather dramatically, it is clear that others were unresponsive.

Clinically, the evaluation of patients with squamous-cell carcinomas of the genital tract is difficult. Unlike patients who have ovarian carcinoma and often have readily measurable parameters, such as ascites or palpable masses, patients with recurrent or persistent squamous-cell cancer often have central disease where regression or progression is difficult to evaluate. Also, only those patients who have received the full extent of conventional therapy in the form of surgery, radiotherapy, or both have been selected for therapy. Another limitation of the current regimen is the exclusion of patients in whom there was significant impairment of renal or hepatic function since the frequency of toxic reactions would be unacceptable.

Although these studies indicate that rational, programmed chemotherapy can, in some instances, modify the progression of squamous-cell cancer, this is a preliminary report where a limited number of drugs in a select group of patients has been utilized *and is not recommended for standard clinical usage.* It is our aim to continue these investigations of rational chemotherapy based on continuing research in the cell kinetic and pharmacokinetic characteristics of the tumor cell population.

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Discussion

Dr. George D. Wilbanks, Chicago, Illinois. It is exciting to read the second installment of this interesting work being done by Averette and his group in Miami. When one analyzes the amount of man hours involved in this study one understands the 5 year gap since the study of normal tissue kinetics of 1970. This is a key work in three important areas; first, the basic cell kinetics of normal epithelium, dysplasia, carcinoma in situ, and invasive squamous-cell carcinoma of the cervix and vulva; second, the effectiveness of local infusion vs. systemic chemotherapy in recurrent squamous-cell cancers; finally, and perhaps the most important practicality, the use of these cell kinetic observations in the programming of chemotheraputic drug regimens for the treatment of gynecologic cancers.

In spite of a wealth of data on cell kinetics from in vitro systems and from animal in vivo systems, little information has been accumulated in human in vivo systems because of the problem of the amount of radioactive material needed to label the cells. In a recent report using somewhat different techniques, Nitze, Ganzer, and Vosteen[1] in Frankfurt studied the cell kinetics of patients with ENT squamous lesions and used these data in programming radiation therapy. Their study corroborates most of the observations of Dr. Averette. They found that both normal and malignant tissues could be synchronized equally well and that after synchronization no differences occur between normal and malignant tissues in the timing of the cycle phases. They also had a remarkable 50 per cent long-term survival rate of these advanced lesions. Averette found rather similar times in S, G_2, and M phases of the cycle of normal and malignant cells, with the difference in the total cycle time found between normal and malignant cells being a decrease in the G_1 phase in the malignant cells. Two interesting ancillary observations made in this study have been supported by in vitro observations on human cervical epithelium in both Richart's and our laboratories. These are that CIS seems to behave in a manner intermediate between normal and invasive tissues and that the time in mitosis is longer in CIS than in normal epithelial cells. Averette's calculated time in mitosis is shorter than that observed by others in similar studies and in our actual

observations by time lapse cinematography in vitro. This is a minor point. The more important times of S and G_2 are consistent with other studies in the literature.

Averette's studies were performed in the surfaces of the tumors and from specific areas. Have samples been taken from any metastatic lesions to see if these cells behave similarly and do samples from several areas of the same tumor behave in a similar fashion?

The question of the effectiveness of local infusion vs. systemic chemotherapy apparently is still open from the data presented. Is Dr. Averette's group still using arterial infusion? His results of the two long-term cures with this technique, and the relatively low complication rate presented, seem somewhat at odds with other observations in the literature. Do the authors have any data on infusion therapy not given using the kinetic techniques for comparison? Was the three-drug regimen used on the last 15 patients more effective than the initial one- or two-drug regimens?

The use of the programming of systemic chemotherapy with the cell cycle kinetics seemed to be effective in the tissue studies. The survival curves of those patients that responded seem different from those that did not respond or had no treatment. Would Dr. Averette comment on any statistical analysis of this difference?

Finally, I think Dr. Averette and his group should be commended for their persistence in this important work. We, as gynecologists, should capitalize on this work and institute programmed studies in the use of both chemotherapy and radiation therapy on less advanced gynecologic malignancies in a large cooperative study.

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DR. JAMES H. NELSON, JR., Brooklyn, New York. The cell generation time method which Dr. Averette has described concerns me from a technical standpoint. First of all, a 0.1 mm. fluid volume is quite small and a 4 mm. punch biopsy is relatively large. Could Dr. Averette be certain of the diffusion of the injected fluid and did he take more than one biopsy from the same cervix or the same tumor after a single injection?

I realize that with the long half-life of tritium one could not afford to inject the material intravenously or intra-arteriorly in any significant amount.

A fine point in the description of technique: I wonder why Dr. Averette did not infuse saline between courses to maintain patency of the catheter rather than a glucose solution. Glucose carries greater risk of infection, but also saline would prevent obstruction of the catheter by a clot.

What is the dose of methotrexate actually infused? The 15 mg. given in the paper I received does not seem to be a very high dose, since doses higher than that are routinely used in the treatment of malignant trophoblastic lesions. There was also a contradictory statement in the paper concerning how often courses were given. On one page it was stated that a course was repeated every 48 hours, while on the next page it was indicated that the course of therapy was repeated every 3 weeks.

The difference between groups of responses seems too finely drawn to be accurate. For example, the difference between a 50 per cent reduction in size of the tumor and 25 per cent reduction in size seems as though, at the very best, it would have to be an estimate. Of course, it may be that Dr. Averette is measuring this by some more objective means, such as sonography, but no mention was made in the paper of such methods.

As one reads the results, the success rate is not overwhelming but it must be remembered that the patients selected for this study had all received conventional therapy and were classified as terminal patients or patients for whom no other therapy was available. When placed in that context, even two long-term survivors of 7 and 8 years must be viewed with some interest and, indeed, they are impressive.

A complication mentioned in one of these long-term survivors was that of vaginal stenosis and I wonder if Dr. Averette thought that it was due to the chemotherapy?

It occurred to me as I read this paper that most of the patients had had radiation therapy, which we all know is an extremely destructive form of treatment. Dr. Averette himself mentioned the fact that due to the fibrosis secondary to radiotherapy it may be that the chemotherapeutic agents were not actually delivered to the tumor bed in concentrated levels. In the late 1950's and early 1960's, when we were doing a lot of intra-arterial chemotherapy, fluorescein was injected through the catheter and the area supplied by the artery injected was viewed with an ultraviolet light to see if the area fluoresced. In this way one could determine whether or not the catheter was properly placed and also whether, in fact, the arterial system was patent. If the area fluoresced one could be certain the agents to be infused would reach the tumor under treatment. Dr. Averette does not mention this anywhere in the paper and I wonder if he has used this method to determine whether or not his agents are getting to the tumor. If he has not done that, I would recommend it to him in the future because it might very well correlate with the ultimate response. In other words, it might explain in part why some patients are complete failures with progression of the tumor under treatment while others give good remissions.

The approach is well thought out and the work carefully done. While I might question the definitions

of response, an attempt has been made to document the results in an objective manner. The attempt to use chemotherapy in a more rational way is also a most desirable step in the right direction.

DR. BRUCE H. DRUKKER, Detroit, Michigan. Dr. Averette and his colleagues' detailed study moves the modality of chemotherapy to a practical area.

Two question arise, one speculative and the other inquiring.

Is it feasible to consider dose reduction with triple therapy based on the high cell labeling index seen following methotrexate treatment? Potentially this would produce less alteration in the patient's remaining immunity as well as reduction in the toxic side effects. It would appear that the dosage of the chemotherapeutic agents utilized in the study is equal to that which would be utilized if each was used alone.

Second, although this was not mentioned in the paper, has Dr. Averette had an opportunity to study any patients who had been given adriamycin by means of autoradiography? On the basis of Dr. Pattillo's work, one might find a high degree of labeling with adriamycin.

DR. AVERETTE (Closing). Drs. Nelson and Wilbanks raised the question regarding possible cell kinetic differences between primary tumors and metastatic lesions. Thus far, we have found no difference between primary cervical squamous cancer that has extended to the vulva or vulvar carcinoma that has extended to the groin. I think it is important to recognize that what we are measuring really is a rough estimate of the average cell generation cycle in an asynchronous cell population. Also, it was questioned whether or not we found differences in kinetic activity in different parts of the same tumor. With our local injection technique we infiltrated the tumor in multiple areas and we found no uptake of labeled thymidine in areas of necrosis, as might be expected. Also, in areas where there apparently were viable tumor cells, we could detect no uptake in some areas and active incorporation in other areas.

Possibly the injected thymidine did not reach all of the tumor cells, or the viable nonlabeled cells were in the G_0 or nonactive clonogenic population. However, our studies have been confined to areas within the tumor showing active uptake of thymidine. This seems reasonable, since our aim is to study the actively dividing tumor cells so that we can program therapy based upon the proliferative compartments within the tumor.

With respect to systemic vs. infusion chemotherapy, I do not think we have treated enough patients to determine which technique is best. Certainly infusion is more complicated, requiring hospitalization for prolonged periods. In general, large tumors confined to the pelvis were treated by pelvic infusion and patients with disseminated disease were treated systemically.

Dr. Nelson raised the question of vaginal stenosis in the patient treated first for vaginal carcinoma by radiotherapy and later by infusion chemotherapy. I think there is no doubt that stenosis was due to radiotherapy since she was treated rather radically with radiation prior to recurrence and chemotherapy.

We have not used fluoroescence to determine infusate distribution. Instead, we utilize pelvic arteriography as well as a scan of the pelvis following a slow drip infusion of macroaggregates of radioiodinated human serum albumin. Unfortunately, at times we do find poor infusion in areas where there is active tumor associated with extensive radiation fibrosis.

Drs. Wilbanks and Nelson commented on our methods for evaluating clinical response to chemotherapy. Certainly it is difficult to accurately judge objectively and subjectively response to treatment, especially with squamous carcinoma of the pelvis. Usually we did not have large tumor masses or ascites that could be measured as one might do with ovarian cancer. However, we made the best observations that we could with the disease that we were treating. Our method for analysis of the survival curve was based on the life-table method of Cutler and Edderer.

Dr. Drukker mentioned the possibility of reducing the total dose of drugs after initial synchronization. This might be possible. However, severe toxicity occurred usually in the early phase of the study when we treated patients with compromised renal or hepatic function. Adriamycin has been used on several occasions recently but we have no conclusions regarding the drug at this time.

Cytology and colposcopy in the diagnosis and management of preclinical carcinoma of the cervix uteri: A learning experience

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PETRA BANOGAN, M.D.
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New York, New York

This report presents a learning experience in the use of colposcopy in the management of 254 patients consecutively and personally studied in whom one or more Papanicolaou smears suggested a cervical intraepithelial neoplasia or early invasive carcinoma. The accuracy of cytology in predicting the degree of cervical intraepithelial neoplasia and early invasive carcinoma, within the limits of confidence of the laboratory, was 84.5 per cent. The accuracy of the colposcopic impression, within the limits of confidence, was 83.5 per cent. When only those lesions that could be satisfactorily examined were considered, the accuracy of the colposcopic interpretation within the limits of confidence was found to be 96.4 per cent. Neoplastic lesions limited to the ectocervix, and lesions that extended from the ectocervix into the lower portion of the endocervical canal and lesions limited to the canal were significantly related to the age of the patient. Of the total group of 254 patients examined, 45 patients (17.7 per cent) were subjected to a diagnostic cone biopsy.

THE INCREASING accuracy achieved by exfoliative cytology over the past two decades in predicting epithelial neoplastic changes in the uterine cervix is well recognized. As a result of this phenomenon the increased use of the diagnostic cone biopsy to obtain a histologic diagnosis has also occurred. It has been well established, however, that a cone biopsy is not to be regarded as a benign procedure since it is often associated with a significant degree of surgical morbidity and jeopardy to future childbearing. Moreover, when a diagnostic cone biopsy is performed because of a suspicious or positive Papanicolaou smear, in the absence of any gross or suspicious overt lesion on the cervix, the incidence of finding an invasive carcinoma requiring radical therapy is small, usually in the range of 4 to 5 per cent.[1–4]

With the introduction of the colposcope in this country and of exfoliative cytology in Europe over 20 years ago, reports comparing the effectiveness of the combined use of these two methods of screening for cervical cancer began to appear in the literature.[5–11] These comparative studies substantiated that it was possible to pinpoint accurately the area of abnormal cervical epithelium by colposcopic examination for the selection of the biopsy site.

In 1971 Coppleson and associates[12] published a classic monograph entitled *Colposcopy* and a year later Kolstad and Stafl[13] published a superb *Atlas of Colposcopy*. Thus there appeared for the first time in the more recent English literature two authoritative treatises on colposcopy and its application in the understanding and diagnosis of cervical neoplasia from which the interested gynecologist could learn the basic principles involved in the clinical application of colposcopy.

The purpose of this report is to present our learning

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experience in the first 3 years in the combined use of cytology and colposcopy in the diagnosis of cervical intraepithelial neoplasia (CIN) and early invasive carcinoma and to evaluate the individual and combined accuracy of these well-established modalities in predicting the histologic diagnosis of cervical dysplasias, carcinoma in situ, and early invasive carcinoma of the cervix. The management and end results of the first 254 patients consecutively seen and personally studied in a dysplasia clinic, in whom a cytologic diagnosis suggestive of cervical dysplasia, carcinoma in situ (CIS), microinvasive carcinoma (MIC), or invasive carcinoma (IC) was made, will also be presented.

Material and methods

Dysplasia clinic, selection of patients, and population group. In September, 1971, a weekly cervical dysplasia clinic was organized as part of the Tumor Service of Woman's Hospital, to which all patients with a grossly benign cervix and at least one and usually two or more Papanicolaou smears suggestive of cervical intraepithelial neoplasia or invasive cancer were referred for colposcopic examination and evaluation.

The patients served in this clinic were from an inner city population group. Less than 6 per cent of the patients comprising this report were private patients referred by members of the attending staff. The cytologic profile of the population served in the general obstetrics and gynecology clinics is shown in Fig. 1. The majority of patients were derived from the group of patients in whom a "suspicious" or "positive" Papanicolaou smear had been obtained.

Colposcopic examination. Each colposcopic examination included a repeat smear, the application of 3 per cent acetic acid to the cervix, the use of the green filter and a colposcopic magnification of 16×, or more when indicated, as well as the use of the Schiller stain to the cervix and upper vagina when the colposcopic findings were grossly at variance with the cytologic impression.

Histologic diagnosis. Final histologic diagnoses were obtained by either a colposcopically directed biopsy or a cone biopsy, or both. If the upper limits of a colposcopic abnormal area extended into the canal, beyond the limits of the examination, the patient was admitted to hospital for a repeat colposcopic examination, including a Schiller test, a cone biopsy, and a fractional curettage in the operating room. An endocervical curettage (ECC) was rarely performed in this study because of the inability to distinguish with sufficient accuracy the depth of invasion of the neoplastic process, if it existed, and the prolonged time

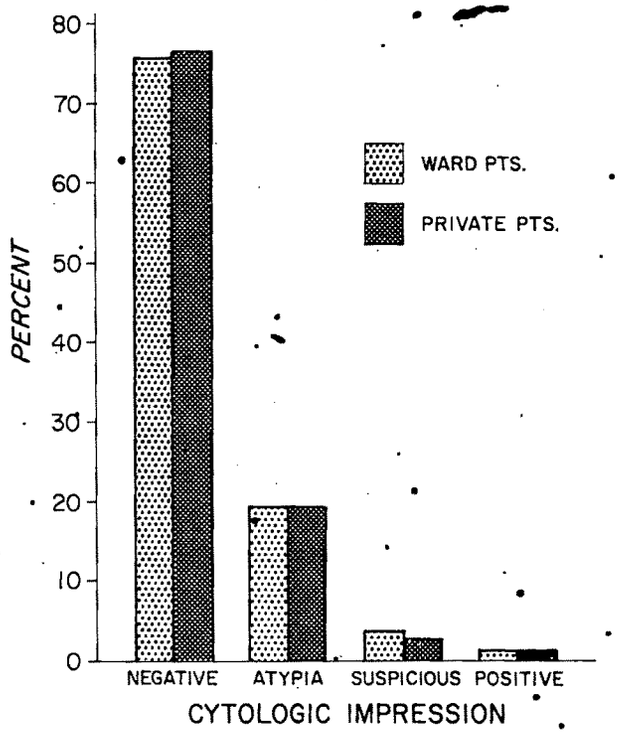


Fig. 1. The cytologic impression in 42,803 cervical Papanicolaou smears examined in a 4 year period from 1971 to 1974.

required for the endocervical epithelium to regenerate before a useful diagnostic cone could be performed.

Cone biopsies were processed by the method described and perfected by Burghardt[14] but modified to suit the demands of a busy, routine diagnostic laboratory.

Management protocol. All patients with an abnormal cervical cytology were managed according to a previously established protocol based on a preliminary study of 135 patients,[4] and outlined in Fig. 2. The definitive treatment of patients with a minor or major dysplasia, CIS or MIC was occasionally modified to satisfy the emotional or obstetric needs of the patient rather than the specific histologic diagnosis.

Definitions and limits of confidence. The *cytology* reports of the Papanicolaou smears included both a descriptive interpretation and a coded cytologic impression of the most probable histologic diagnosis. Minor dysplasia, major dysplasia including CIS or MIC, and invasive carcinoma were the three major degrees of neoplasia that could be confidently distinguished cytologically. When certain obvious, more specific, and identifiable cytologic features were present they were so described and a more precise impression of the probable histologic changes were coded.

The diagnosis of the histologic continuum of epithe-

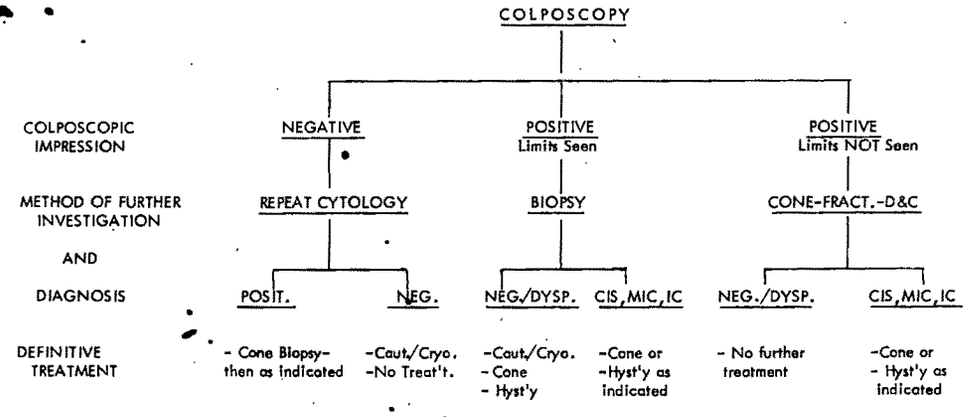


Fig. 2. Outline of management of patients with abnormal cervical cytology.

Table I. Frequency of observed colposcopic features in 125 patients whose colposcopic impression agreed precisely with histology of directed biopsy

	Colposcopic impression and histology of directed biopsy					
	Benign–min. dysp. (N = 62)		*Maj. dysp.–C.I.S. (N = 37)*		*M.I.C.–I.C. (N = 26)*	
Colposcopic feature	*No.*	*%*	*No.*	*%*	*No.*	*%*
Focal white lesion	40	64.5	16	63.0	17	65.4
Surface flat	62	100.0	21	56.7	2	7.7
Surface irregular	0	0	7	19.0	12	46.2
Atypical B. V.	0	0	6	16.2	23	88.5
Mosaicism regular	5	8.0	0	0	0	0
Mosaicism irregular	4	6.4	9	24.4	4	15.4
Mosaicism mixed	10	16.1	17	46.0	18	69.2
Punctation fine	19	31.0	5	13.5	1	3.8
Punctation coarse	2	3.2	2	5.4	2	7.7
Punctation mixed	14	22.5	19	51.3	14	54.0

lial changes associated with CIN were based on the same morphologic criteria accepted by most pathologists. Both the degree of severity and the relative extent of the cellular changes involving a given segment of epithelium formed the basis for providing a semiquantitative and arbitrary division of CIN into mild, moderate, and severe dysplasia, culminating in CIS where the entire thickness of the epithelium was occupied by frankly neoplastic cells. It is emphasized that the neoplastic cells in CIS may display varying degrees of maturation in the form of keratinization. This concept has been widely recognized[14–16] and is at variance with an earlier definition of this lesion.[17]

No strict histologic definition of MIC has been attempted since a wide range in the acceptable depth of stromal infiltration exists.[18–20] Our own criteria tend to be more conservative, limiting the depth of acceptable invasion closer to 1 mm. as suggested by Nelson and associates.[18]

It is not always possible with *colposcopy* to confidently predict the precise degree of epithelial neoplasia by the appearance of the abnormal epithelium. Because of this imprecision, particularly noted in the lesser degrees of neoplasia, five colposcopic diagnoses were confidently recognized based on the observed features. *Minor dysplasia* implied a histologic diagnosis of a mild, or mild to moderate, or moderate dysplasia, and a *major dysplasia* implied a histologic diagnosis of moderate to severe, or severe dysplasia. A colposcopic diagnosis of *CIS* implied either a histologic severe dysplasia with foci of CIS or an unqualified CIS. Colposcopic diagnosis of *MIC* and *IC* implied the same as their histologic counterpart.

A correct colposcopic diagnosis within the limits of confidence permitted the inclusion of one degree more or less of histologic neoplasia.

Results

Age of patients. The mean age of the total group of 221 patients with CIN, MIC, and IC was 33.6 years.

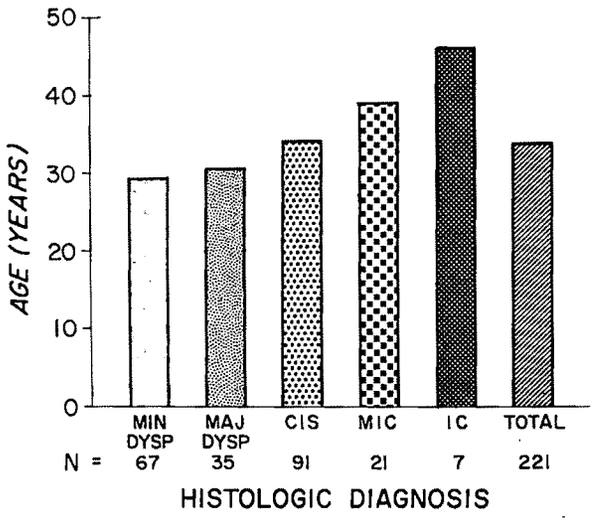


Fig. 3. Mean age of 221 patients with CIN and IC grouped according to histologic diagnosis.

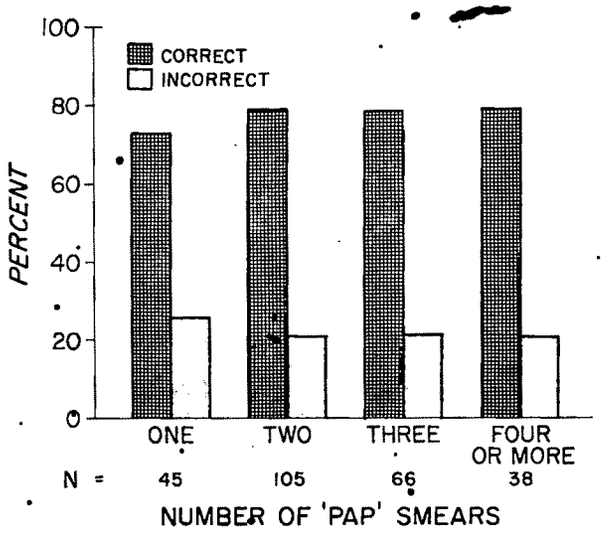


Fig. 4. The accuracy of the cytologic impression related to the number of abnormal Papanicolaou smears (254 patients).

Table II. Management and end results of primary treatment of 214 patients with CIN and MIC

Management	Cytology (at 6 mo.) Negative	Cytology (at 6 mo.) Abnormal	LFU* (at 6 mo.)
Minor dysplasia:			
Biopsy only (N = 17)	10	2[a,b]	5
Cautery/cryo. (N = 41)	39	1[a]	1
Cone biopsy (N = 9)	8	0	1
Total (N = 67)	57 (85.0%)	3 (4.5%)	7 (10.5%)
Major dysplasia:			
Biopsy only (N = 3)[c]	1	1	1
Cautery/cryo. (N = 15)	12	2[d]	1
Cone biopsy (N = 15)	14	1[e]	0
Hysterectomy (N = 2)	2	0	0
Total (N = 35)	29 (82.8%)	4 (11.4%)	2 (5.8%)

*LFU, lost to follow-up.

[a]Two patients, cone at 3 months, minimum dysplasia.

[b]One patient, refused treatment.

[c]Three patients, refused treatment.

[d]Two patients, cone biopsy at 4 months.

[e]One patient, negative cytology at 5 months, abnormal cytology at 6 months.

[f]Four patients, refused treatment.

[g]Two patients, abnormal cytology at 3 and 4 months; hysterectomy; no CIN.

[h]Two patients, abnormal cytology at 2 and 4 months when LFU.

[i]Three patients, refused treatment.

Table II—cont'd

Management	Cytology (at 6 mo.) Negative	Cytology (at 6 mo.) Abnormal	LFU* (at 6 mo.)
CIS:			
Biopsy only (N = 4)	—	—	4[f]
Cautery/cryo. (N = 6)	4	2[g]	0
Cone biopsy (N = 35)	30	0+?	5[h]
Hysterectomy (N = 46)	36	0	10
Total (N = 91)	70 (76.9%)	2+ (2.2%+)	19 (20.9%)
MIC:			
Cone only (N = 3)[i]	3	0	0
Hysterectomy (N = 18)	18	0	0
Total (N = 21)	21 (100.0%)	0	0
Total (N = 214)	177 (82.7%)	9+ (4.2%)	28 (13.1%)

Fig. 3 shows the mean age of patients according to the category of neoplasia and indicates the increase in mean age with each increase in degree of neoplasia.

Colposcopic features and their significance in establishing a colposcopic impression. The more commonly observed colposcopic features and the frequency with which they were seen, according to the degree of histologic neoplasia in 125 patients whose colposcopic impression agreed precisely with the histologic diagnosis, are shown in Table I. Flat white

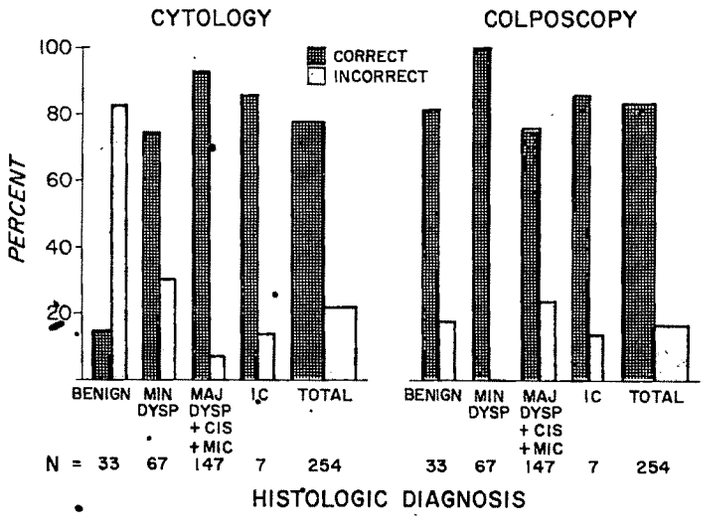


Fig. 5. Comparative accuracy of cytology and colposcopy in the diagnosis of CIN and IC in 254 patients with abnormal Papanicolaou smears.

lesions with no abnormal surface or blood vessels, or that showed a regular mosaic pattern with or without fine punctation, usually indicated a benign or minor dysplastic lesion. White lesions with an irregular surface and abnormal, nonbranching, surface blood vessels, with or without a mixed mosaic pattern or a mixed type of punctation, were associated more frequently with the more advanced degrees of neoplasia. Nonbranching blood vessels running parallel along an irregular, densely white epithelium were the most consistently observed features associated with a microinvasive or invasive carcinoma.

Accuracy of cytologic impression. The Papanicolaou smear correctly predicted within the limits of confidence of the cytologist the histologic diagnosis in 198 (77.8 per cent) of the total group of 254 patients. Of the 221 patients with dysplasia, CIS, MIC, or IC, however, the cytology report correctly predicted the histologic diagnosis in 84.5 per cent of patients. Thirty-three patients had a benign cervical lesion, 28 of whom had a cytologic impression consistent with a minor or major dysplasia. Most of these smears were noted to have an inflammatory type of cellular atypia that was indistinguishable from dysplastic cellular changes.

Fig. 4 indicates a 5 per cent increase in accuracy of the cytologic impression between the first and second abnormal smear. No apparent increase in the accuracy of the Papanicolaou smear occurred with subsequent smears.

Accuracy of colposcopic impression. Of the 254 patients examined, the colposcopic impression, within the limits of confidence, agreed with the histologic diagnosis in 212 patients (83.5 per cent) and precisely agreed with the histologic diagnosis in 114 patients (53.8 per cent). In 34 patients the lesion was either limited to the endocervical canal (17 patients) or extended far into the canal beyond colposcopic vision (17 patients). If these unsatisfactory examinations were excluded from the analysis the colposcopic impression in the remaining 220 patients examined would be 96.4 per cent correct.

The comparative accuracy of the cytologic and colposcopic impressions, grouped according to the three degrees of neoplasia confidently recognized cytologically, are seen in Fig 5.

Colposcopic site of lesion. The frequency with which the four degrees of colposcopic neoplasia were found on the ectocervix or in the canal, or both, is seen in Fig. 6. The percentage distribution of the four degrees of neoplasia in the 103 patients whose lesions were limited to the ectocervix and the 94 patients whose lesions extended into the lower canal was significant ($\chi^2 = 41.47$; $p < 0.01$).

The difference in the ages of the patients whose lesions were limited to the ectocervix, and whose lesion extended into the canal, and whose lesions were limited to the canal, were also found to be significantly different ($p < 0.01$). These data are shown in Fig. 7, where a gradual shift toward an older age group of patients is observed when the lesion is limited to the endocervical canal.

Cytologic, colposcopic, and histologic diagnosis of CIN and MIC in pregnancy. There were 31 pregnant

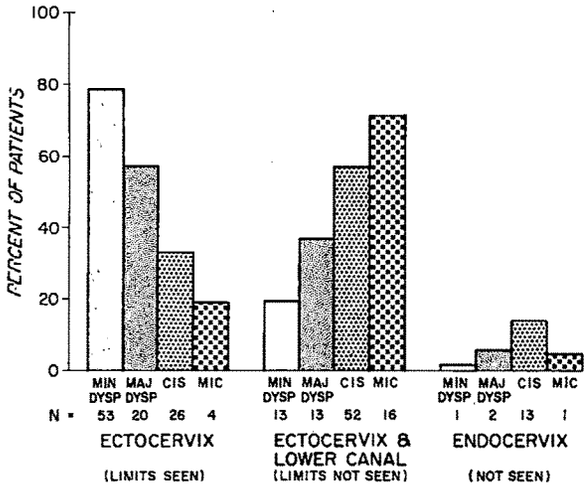


Fig. 6. Site of lesion as determined by colposcopic examination in 214 patients with CIN and MIC.

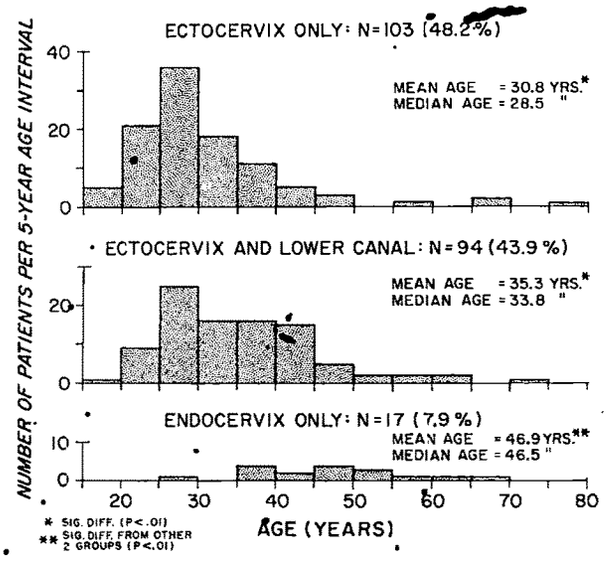


Fig. 7. Age distribution, mean age, and median age of 214 patients with CIN and MIC grouped according to site of lesion.

patients in the total group of 254 patients whose Papanicolaou smear indicated an intraepithelial or early invasive neoplastic process. Cytologically eight patients were overdiagnosed and one patient was underdiagnosed by two or more degrees of neoplasia. The Papanicolaou smears in the remaining 22 patients (71.0 per cent) were correctly interpreted within one degree of histologic neoplasia. The colposcopic lesion in four patients was underinterpreted by two or more degrees of neoplasia and interpreted correctly within one degree of neoplasia in the remaining 27 patients (87.1 per cent).

Management and end results

The management and end results of the 214 patients with CIN and MIC are outlined in Table II. Minor dysplastic lesions were definitively treated with cryosurgery in preference to cauterization in the majority of patients in this study.

Major dysplastic lesions were similarly treated but with a more liberal use of cone biopsy when there was any doubt of the extent or possible degree of neoplasia.

Carcinoma in situ was treated preferentially by therapeutic conization or extrafascial abdominal hysterectomy or vaginal hysterectomy, depending upon the patient's age and her reproductive desires. Six patients accepted only cryosurgery as their treatment, two of whom later accepted a hysterectomy at 3 and 4 months, respectively.

Patients with microinvasive cancer were invariably recommended to have a hysterectomy. Three patients underwent a therapeutic conization for what was thought to be CIS and were found to have MIC in the specimen. All three refused hysterectomy with the knowledge that all margins of the cone were free of the disease.

Patients who continued to have an abnormal Papanicolaou smear 6 months after treatment were considered to be still at risk and not "cured" of their disease. The absolute "cure" rate in the group of 214 patients with cervical dysplasia, CIS, and MIC, was achieved following initial treatment in 177 patients (82.7 per cent). There were 181 patients who accepted the treatment according to the protocol established for this study and 159 of these patients (87.9 per cent) were cytologically negative at 6 months or more.

The management and end results of the 26 pregnant patients with cervical intraepithelial neoplasia or microinvasive disease are summarized in Table III. An absolute "cure" rate was achieved in 20 patients (76.9 per cent) as determined by a negative cytologic examination at 6 months after treatment.

Cone biopsies. In the total group of 254 patients colposcoped because of cytologic evidence suggestive of CIN or early invasive disease, 77 patients (30.3 per cent) were referred for therapeutic conizations and 45 patients (17.7 per cent) for diagnostic conizations. The type of cone performed and the subsequent management according to the histologic diagnosis are seen in Table IV. Eight patients had their uterus removed following conization because of involved surgical margins in the cone biopsy and the remaining 26 patients underwent a hysterectomy either because of the severity of their neoplasia or for other indications.

Table III. Management of pregnancy and end results of primary treatment of 26 pregnant patients with CIN and MIC

Histologic Diagnosis	Management			Follow-up		
	Pregnancy		CIN-MIC Rx./No. Pts.	Cytology		Lost to follow-up
	Abort.	Not abort.		Neg.	Abn'l.	
Minor dysplasia (N = 8)	1	7	Caut.—2/cryo.—3	6[a]	0	2[b]
Major dysplasia (N = 7)	2	5	Cryo.—3/hyst.—2	5[c]	1[d]	1[b]
CIS (N = 8)	1	7	Cone—2/hyst.—6	6	1[e]	1
MIC (N = 3)	2	1	Hyst.—3	3	0	0
Total (N = 26)	6	20		20 (76.9%)	2 (7.7%)	4 (15.4%)

[a]Two patients, no treatment; spontaneous regression.
[b]One patient, no treatment.
[c]One patient, no treatment; spontaneous regression.
[d]One patient, cone biopsy 4 months after cryosurgery, in follow-up.
[e]One patient, only cone biopsy in pregnancy (4 months); margins not free "minor dysplasia"; refused treatment.

Table IV. Type of cone biopsy and number of subsequent hysterectomies performed for disease in 122 patients with CIN, MIC and IC

Cone biopsy diagnosis	Therapeutic cone		Diagnostic cone		Total cone biopsies
	No.	Hysterectomy for disease	No.	Hysterectomy for disease	
Minor dysplasia	5	0	5	0	10
Major dysplasia	12	1	6	2	18
CIS	54	10	21	5	75
MIC	6[a]	5	12[a]	10	18
IC	0	0	1	1	1
Total	77 (63.1%)	16 (20.7%)	45 (36.8%)	18 (40.0%)	122

[a]Three patients, refused hysterectomy.

Comment

This study has emphasized the value and accuracy of colposcopy in determining the degree and site of preclinical cervical neoplasia that has been suggested by an abnormal Papanicolaou smear. When used as an intermediate diagnostic step between cytology and tissue diagnosis it is capable of refining the clinician's evaluation and therapeutic management of patients with early neoplastic lesions of the cervix.

The colposcopic impression in the group of 254 patients was grossly overdiagnosed in 6 patients (2.3 per cent) and underdiagnosed in 36 patients (14.2 per cent). The total error of 16.5 per cent of the colposcopic impressions in this study is comparable to the 15.0 per cent error rate reported in a larger study.[21]

The colposcopic examination of the ectocervix and lower cervical canal is greatly facilitated in pregnancy because of the physiologic eversion of the lower cervical canal that occurs at this time. It is suggested that the greater accuracy of the colposcopic impression in the 31 pregnant patients (87.1 per cent) in this study compared to that of the group of 254 patients as a whole (83.5 per cent) is due to the increased visibility of the epithelium at risk seen in the pregnant patient.

It was of some interest to visually confirm the well-documented colposcopic and colpomicroscopic observations of others[12, 22, 23] that CIN is significantly limited to the transformation zone on the ectocervix in a younger age group and is observed extending into the lower end of the endocervical canal in an older age group and is limited to the endocervical canal in a still older group. The mean age difference between patients with lesions limited to the ectocervix and patients with lesions extending into the endocervical canal was 5.5 years, whereas the mean age difference between the later group and patients with the lesions limited to the canal was 11.6 years.

In the total group of 254 patients, 45 patients (17.7 per cent) were subjected to a diagnostic cone biopsy, of which a few were also therapeutic and 77 patients (30.3 per cent) were treated by a therapeutic cone biopsy,

some of which were also diagnostic. The point to emphasize is that only 122 (48.0 per cent) of patients received a cone biopsy, which is an incidence rate similar to those of other comparable series.[21, 22] Without colposcopy it is estimated that a great majority of the 254 patients would have been subjected to a conization and all the inherent complications associated with this procedure.

The absolute "cure" rate of 82.7 per cent of the 214 patients with CIN or MIC, as determined only for the purpose of this report by at least one negative cytologic examination within 6 months following initial treatment, is undoubtedly influenced by the 28 patients who were lost to follow-up. Four of these patients were among the 11 patients that refused any treatment and presumably still have their disease. Nine patients with a known abnormal Papanicolaou smear, most of whom suggested atypia, are still in follow-up and either have been retreated, have been definitively treated, or have become cytologically negative.

Ideally, we believe that all patients treated for a CIN and MIC should be followed both cytologically and colposcopically every 3 or 4 months for 1 year and every 4 or 6 months in the second year and once a year thereafter. Although our experience has not been as extensive as others, the pattern of treatment and the intensive follow-up care for a minimum of two years, as suggested by more experienced investigators[26] who have observed no recurrence up to seven years in patients who have been free of their disease for 24 months following treatment, seems reasonable.

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Discussion

DR. GEORGE A. HAHN, Philadelphia, Pennsylvania. Dr. Tovell and his co-authors have investigated a group of 254 patients in whom cytologic smears suggested preinvasive or invasive carcinoma of the cervix uteri. They utilized a logical, workable protocol for management, stressing the colposcopic features of whiteness, surface contour, mosaicism, abnormal blood

vessels, and punctation. In those patients where the lesion was completely visualized by the colposcope a creditable 96.4 per cent accuracy was obtained.

My own experience with the colposcope dates back to the early 1950's when Dr. Karl Bolten, from the University of Bonn and a protege of Dr. Heinrich Martius (formerly of Gottingen), came to Jefferson Hospital with the sponsorship of Dr. Lewis Scheffey. In an early publication Scheffey and Lang[1] cited the advantages of colposcopy as (1) rapid screening of the portio of the cervix uteri (2) localization of abnormal epithelial patterns for further analysis by cytologic or histologic means (3) the avoidance in some cases of the "circular" or "cone" type biopsy of the cervix when seemingly benign changes are noted by colposcopic examination in the presence of grossly suspicious areas. The major disadvantages were listed as the expense of the instrument $1,750.00 and the amount of time necessary to perform a proper examination.

In the same era Hahn[2] mentioned that colposcopy is not intended to take the place of cytologic smears or biopsy, but supplement them, serving as a link between clinical appearance of the cervix, cytologic study of cellular material, and histologic examination of excised tissue.

In a study of 6,870 patients Held and associates[3] approximated the time devoted by physicians and laboratory personnel to be 25 minutes per patient for colposcopy and 11 minutes per patient for cytologic screening. In this series of screening examination 39 microinvasive and in situ carcinomas were detected.

Has Dr. Tovell considered the possibility of paramedical aid in colposcopy? I question the use of a 6 month period as a follow-up of patients with cervical neoplasia particularly with a loss of 13.1 per cent of the patients to follow-up.

Surgery, electrocoagulation, and cryotherapy are effective methods of managing cervical dysplasias and in situ and microinvasive carcinona of the cervix. Another mode of treatment which is being used more often is the carbon dioxide laser beam as described by Bellina.[4]

It will be interesting to know the subsequent fate of the patients with carcinoma in situ who still had abnormal smears 6 months after treatment. Further comment in regard to endocervical curettage also seems in order.

Dr. Tovell has demonstrated the research, teaching, and clinical value of a planned colposcopic study in conjunction with cytologic screening and indicated surgical procedure.

In this age, when many medical meetings are engulfed by a plethora of basic chemical and physiologic presentations, it is refreshing to read and hear a sound clinical paper.

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DR. WILLIAM C. KEETTEL, Iowa City, Iowa. The value of cytology in the detection of asymptomatic neoplasia in a normal-appearing cervix has been well substantiated. Until recently conization has been considered an ideal method of obtaining representative tissue for diagnosis, thus eliminating the possibility of inadvertently treating an invasive cervical carcinoma by total hysterectomy. Because of the complications and expense of conization there has been increasing interest in finding an accurate, simple method of obtaining tissue sampling.

Dr. Tovell and his co-authors, as have others, have demonstrated by using a combination of cytology and colposcopic-directed biopsy that the number of conizations can be materially reduced. In their series 48 per cent of the patients received a cone biopsy, 17.7 per cent for diagnostic reasons because the squamocolumnar junction was not visualized, and 30.3 per cent were treated by a therapeutic conization. In a similar study in our department of 432 patients, 27 per cent received a cone biopsy, of which 21.7 per cent were diagnostic. In a teaching setting a colposcopic clinic also provides an ideal method of instructing the residents and medical students in the value of the technique.

There are conflicting figures noted in the paper regarding the accuracy of cytology in predicting histologic changes. In the summary cytology was stated to be 84.3 per cent accurate while in the text of the paper it was reported to be 77.8 per cent. This latter figure would agree with our experience where the degree of accuracy was 73.5 per cent. However, if one includes only the more severe forms of dysplasia the accuracy of cytology in predicting the types of change was 84.5 per cent and in our series 85.8 per cent.

In patients showing cytologic evidence of mild atypia or dysplasia, the colposcopic-directed biopsy is more accurate in predicting the correct histologic change, whereas in patients with the more severe cytologic changes the cytology is nearly as accurate as the biopsy. There seems to be a tendency among some cytologists to over read slides in order to alert the clinician when the cytologic interpretation is distorted by inflammatory changes. In our study of 473 patients, 220 patients were referred to us because of an abnormal cytology. In 72 patients or 32.7 per cent of the series our cytology was interpreted as normal. In 21 patients or 10 per cent our cytology was more severe than the outside interpretation. I wonder if Dr. Tovell would comment if they encountered similar discrepancies in comparing outside cytology with their own?

The authors have made an interesting and extremely important observation that the extension of in-

traepithelial neoplasia into the endocervical canal was related to patient age. The mean age difference between patients with lesions limited to the ectocervix and patients with lesions beginning to extend into the endocervical canal was 5.5 years, while the mean age difference between patients with lesions limited only to the canal was 11.6 years. I wonder if Dr. Tovell can explain why this occurs and also why do certain women of advanced age develop invasive cervical cancer without the antecedent evidence of the previous cervical neoplasia?

I was not impressed with the author's attempt to correlate colposcopic impressions with histologic diagnosis despite the 83.5 per cent over-all accuracy. The colposcopist who has prior knowledge of the cytology has a marked advantage in predicting the type of change. A more accurate way of determining such correlations would be to describe the colposcopic impression without prior knowledge of the cytologic findings. The other area of disagreement is related to the statement that the absolute "cure" rate was 82.7 per cent as determined by at least one negative cytology 6 months following definitive treatment. A much longer period of follow-up is required before one can speak of absolute cure rates. We have seen carcinoma in situ of the vagina or invasive cancer develop 5 to 8 years after treatment for carcinoma in situ.

DR. MICHAEL NEWTON, Chicago, Illinois. I rise as a confirmed enthusiast for colposcopy and a second-class historian. I have had dealings with colposcopy for many years, and one of the functions of discussants in this Society is, I think, to be a historian. Unfortunately, Dr. Hahn upstaged me. Dr. Carl Bolten began a colposcopy clinic under my direction at the University of Mississippi in the early 1960's, and we at that time referred to him all patients with abnormal Pap smears. When I came to Chicago, Dr. Ray Ortiz and later Dr. Sonek have conducted colposcopy clinics on much the same lines that Dr. Tovell describes.

We have noted during a period of years a gradual increase in the accuracy of the colposcopic observations. I think this is important. In the early days colposcopy was, to say the best of it, semirespectable. It is becoming respectable now. As a result, our present colposcopists have shown a great increase in their ability to make specific diagnoses, and pride themselves on not missing major lesions. I would agree with the comments that every resident that goes through a program should learn how to do colposcopy, not a very difficult task under adequate instruction. Our data over the years confirm the fact that conization becomes much less necessary. I think our figures are probably lower at the University of Chicago than have been mentioned. The specific indications for conization, however, should be remembered. It is an operation like any other operation and it has clear-cut indications. If you cannot see the endocervix, we do not believe that endocervical curettage is sufficient and feel that a conization should be done. If there is clear disagreement between the cytologic and the colposcopic findings, then further investigation, usually by conization, has to be undertaken.

Finally, I would like to commend Dr. Tovell for his effort to quantitate the lesions seen under the colposcope. This is, I think, an important endeavor because in the past statements about punctation and mosaicism had not been very easy to document. I would like to ask him one question. In our series of colposcopically studied patients we have noted that the lesions occurred more often on the anterior and posterior lips of the cervix rather than at the lateral angle. I wonder if he made any observations on the site of the lesions that he observed.

DR. FRITZ K. BELLER, Muenster, Germany. I want to congratulate the authors for their unusual approach. The comparative value of cytology and colposcopy was previously evaluated comparatively by detection of malignancies in a certain population by either method alone and combined. I think there is now agreement that by using colposcopic target biopsy one can save a number of patients conization. I do not think we can abandon conization entirely, and I would agree with you that certain indications remain. Dr. Khatamee and myself[1] were the first ones to evaluate target biopsy under the colposcope. We came out with an accuracy of 80 to 85 per cent, a result which was confirmed by many investigators. I feel it is just not good enough if we are missing 10 to 15 per cent of microinvasive or invasive cancers. It was therefore our recommendation that if target biopsy under the colposcope is less than invasive cancer, the patient should have conization to clarify the issue.

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DR. TOVELL (Closing). It must be emphasized that colposcopy does not, and should never compete with exfoliative cytology as a screening method for early cervical neoplasia. However, just as the experienced cytologist can provide a reasonably accurate interpretation of the histologic changes occurring in the cervical epithelium, so can the experienced colposcopist predict, with reasonable accuracy, the histologic changes, as well as their extent, occurring on the ectocervix and in the lower cervical canal.

One purpose of this learning experience was to compare the diagnostic accuracy of cytology with that of colposcopy in the various degrees of cervical intraepithelial neoplasia and early invasive carcinoma. Admittedly, there is a large amount of bias in favor of the colposcopist when he makes his diagnosis based on his examination, when he has the descriptive report of the Pap smear available to him. Many years ago, Professor Navratil and his group[6] simultaneously

studied over 300 patients, both cytologically and colposcopically, and found that both methods were in agreement with the histologic diagnoses in 75 per cent of patients studied.

One can debate that it is not important for the colposcopist to make a diagnosis, and all that he needs to do is to identify the site and the extent of abnormal epithelium for both diagnostic and subsequent therapeutic purposes. We would subscribe to this concept, but would hasten to add that it is much more fun and very much more educational in the understanding of the histopathology of intraepithelial neoplasia, to correlate both the colposcopic and histologic changes.

The question of the two rates of accuracy in our colposcopic diagnosis is both valid and pertinent. The 83.5 per cent accuracy rate applied to the total group of 254 patients studied and, including the 17 patients with lesions confined to the cervical canal, and therefore beyond the range of colposcopic vision. If these 17 patients were eliminated from the analysis, then over 90 per cent of the visible lesions in 237 patients were correctly diagnosed within the limits of confidence of the colposcopist.

The employment of trained paramedical personnel for colposcopic examinations, including directed biopsies has merit in any large scale cancer screening and detection program where a medical manpower shortage exists. Indeed, trained personnel are being successfully employed for this purpose both in Southern California and Wisconsin, and probably in other areas in the country.

We agree that the 6 month follow-up time indicated in this study is too short to completely evaluate treatment. Optimally, all patients should be followed for at least 2 years, or more, if they had invasive disease. It was our policy in this study, and still is, to obtain a Pap smear at 3 and 6 months after treatment. Any patient with an "atypical" or "suspicious" smear during this time was considered to have persisting disease and further evaluation and treatment was recommended. Of the 214 patients with dysplasia, carcinoma in situ, and microinvasive disease, 177 patients (82.7 per cent) were known to have had "negative" Pap smears at 6 months. There were 28 patients (13.1 per cent) who were lost to follow-up before 6 months. Of the remaining nine patients (4.2 per cent) with abnormal cytologic examinations at 3 and/or 6 month follow-up, seven were treated more vigorously such as by a therpeutic cone or hysterectomy, and two patients both initially and persistently, refused any treatment.

The 13.1 per cent group of patients who were lost to follow-up before 6 months are of obvious concern to us.

I am unable to explain why some neoplastic lesions are limited to the endocervical canal, and have had no previously documented abnormal cytologic examination. Colposcopy, as well as colpomicroscopy have taught us that cervical neoplasia is initialed at the junction of the original stratified squamous epithelium and metaplastic squamous epithelium. Presumably, in these cases, the junction of the two types of squamous epithelium is in the endocervical canal.

It was encouraging to learn that other experienced colposcopists share a similar dislike, if not distrust for the endocervical curettage. Not only have we found the tissue samples to be unreliable, but also subject to a great deal of misinterpretation. On at least two occasions, we underdiagnosed the lesion and subsequently undertreated the patient. We would much prefer to perform a diagnostic cone biopsy and fractional curretage, with the knowledge that in some cases, the procedures will also be therapeutic.

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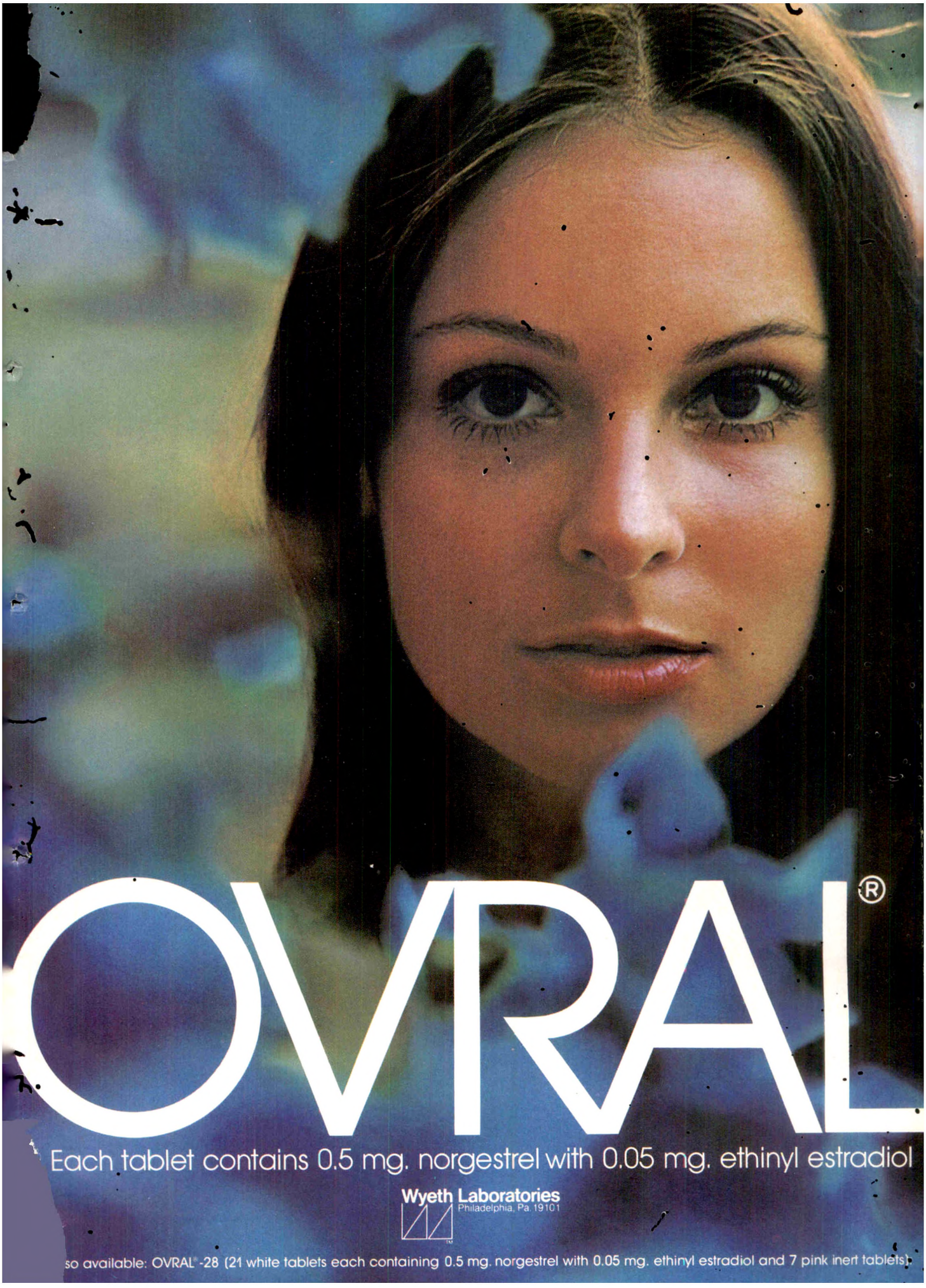
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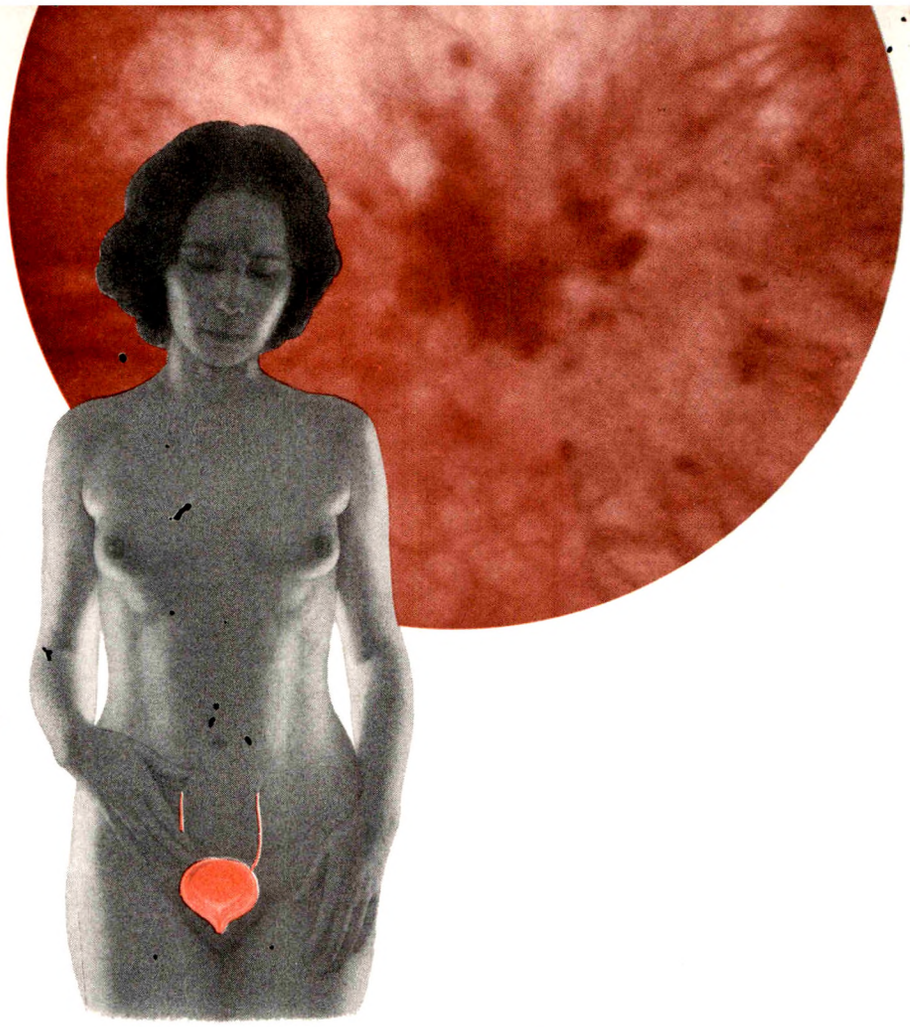
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Warnings: Safe use in pregnancy has not been established. Teratogenicity potential has not been thoroughly investigated. Deaths from hypersensitivity reactions, agranulocytosis, aplastic anemia and other blood dyscrasias have been reported; clinical signs such as sore throat, fever, pallor, purpura or jaundice may be early indications of serious blood disorders. Complete blood counts and urinalysis with careful microscopic examination should be performed frequently during sulfonamide therapy.

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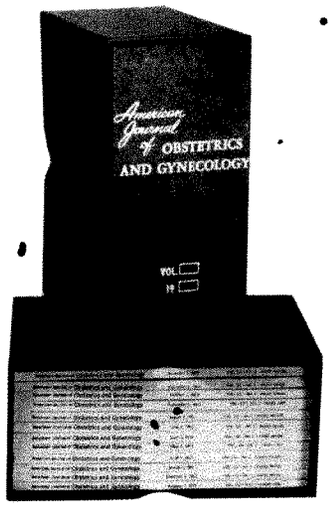
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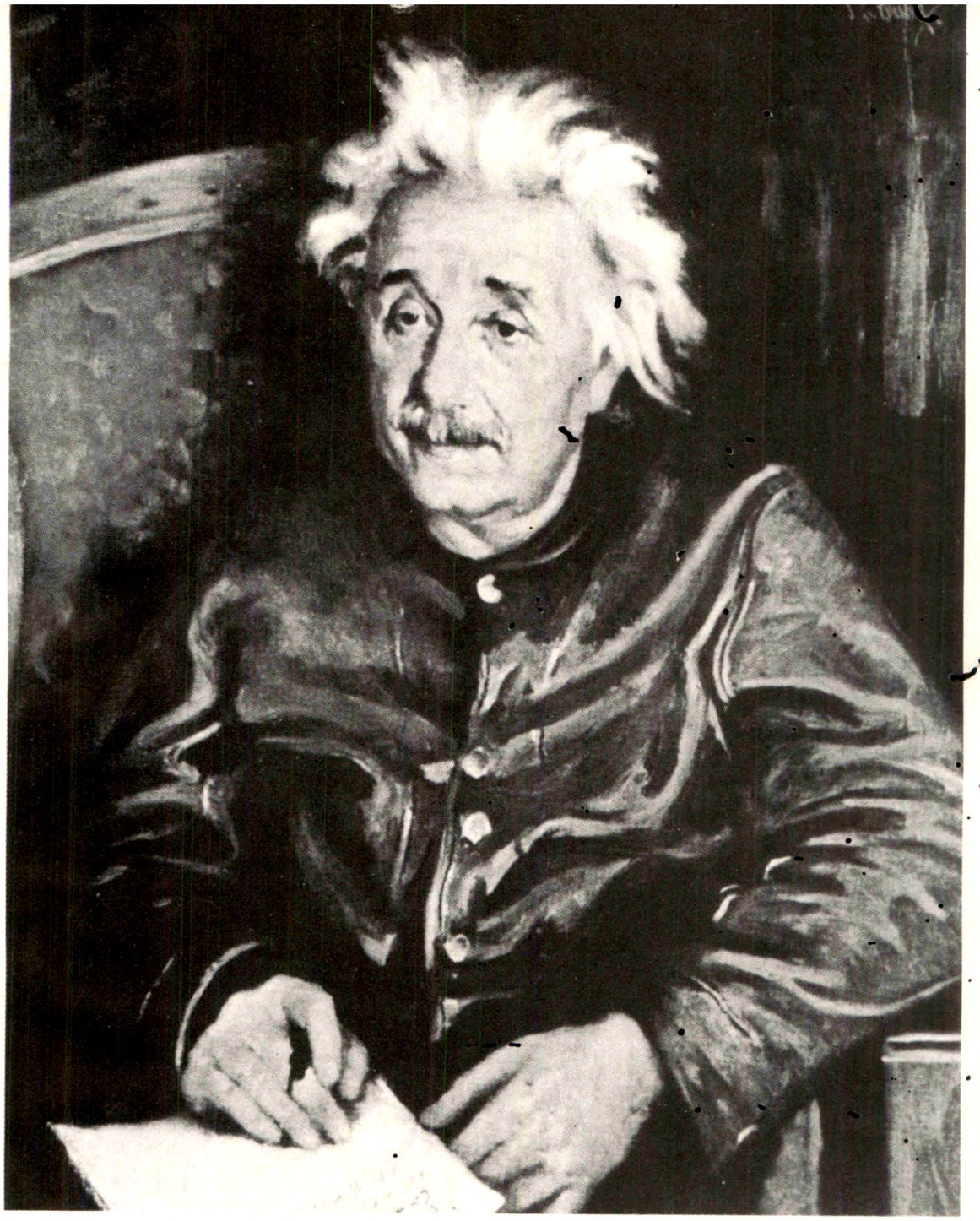
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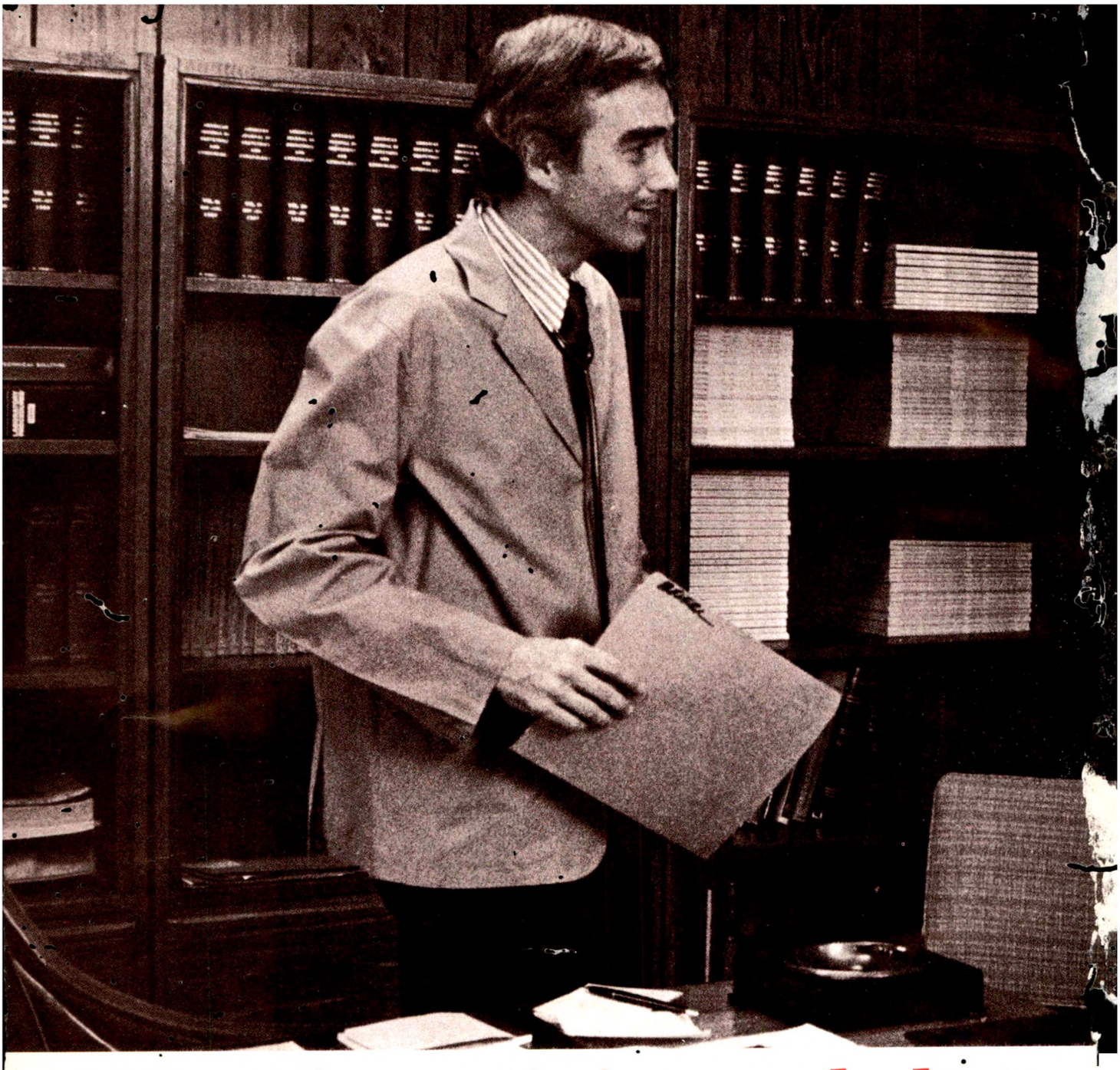
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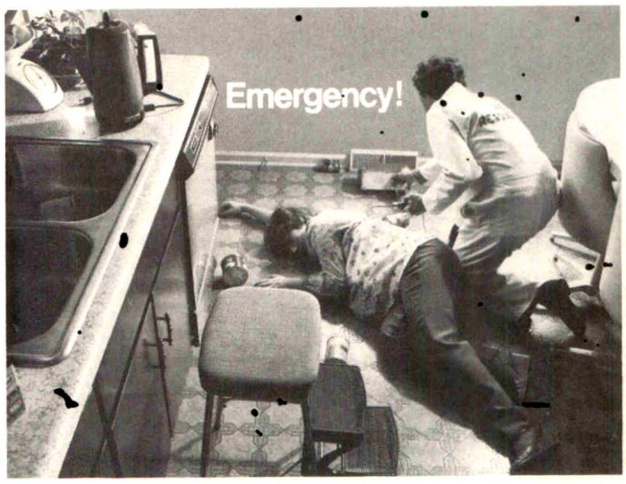
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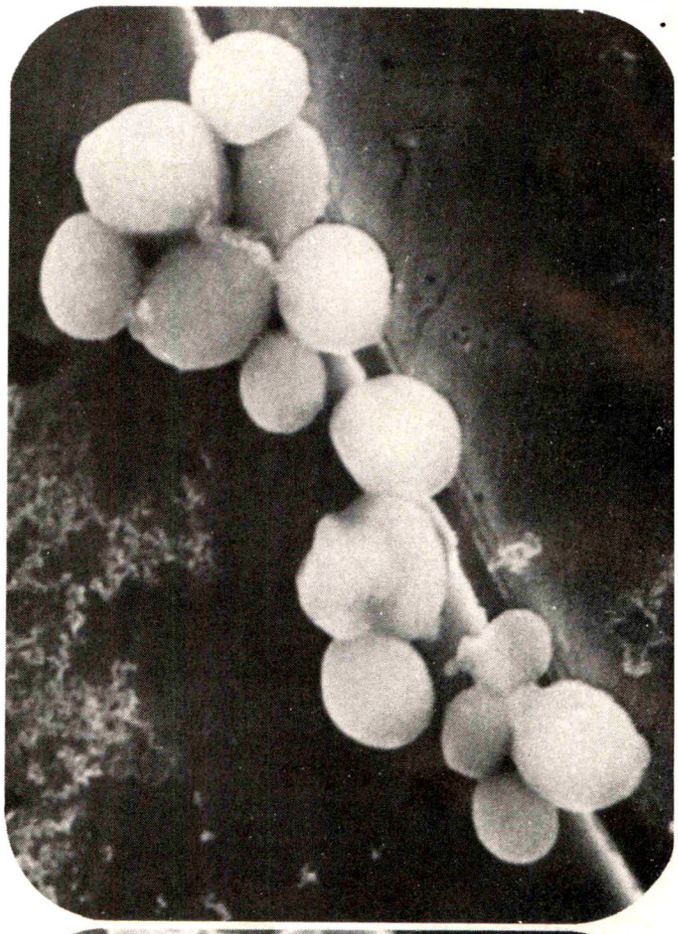
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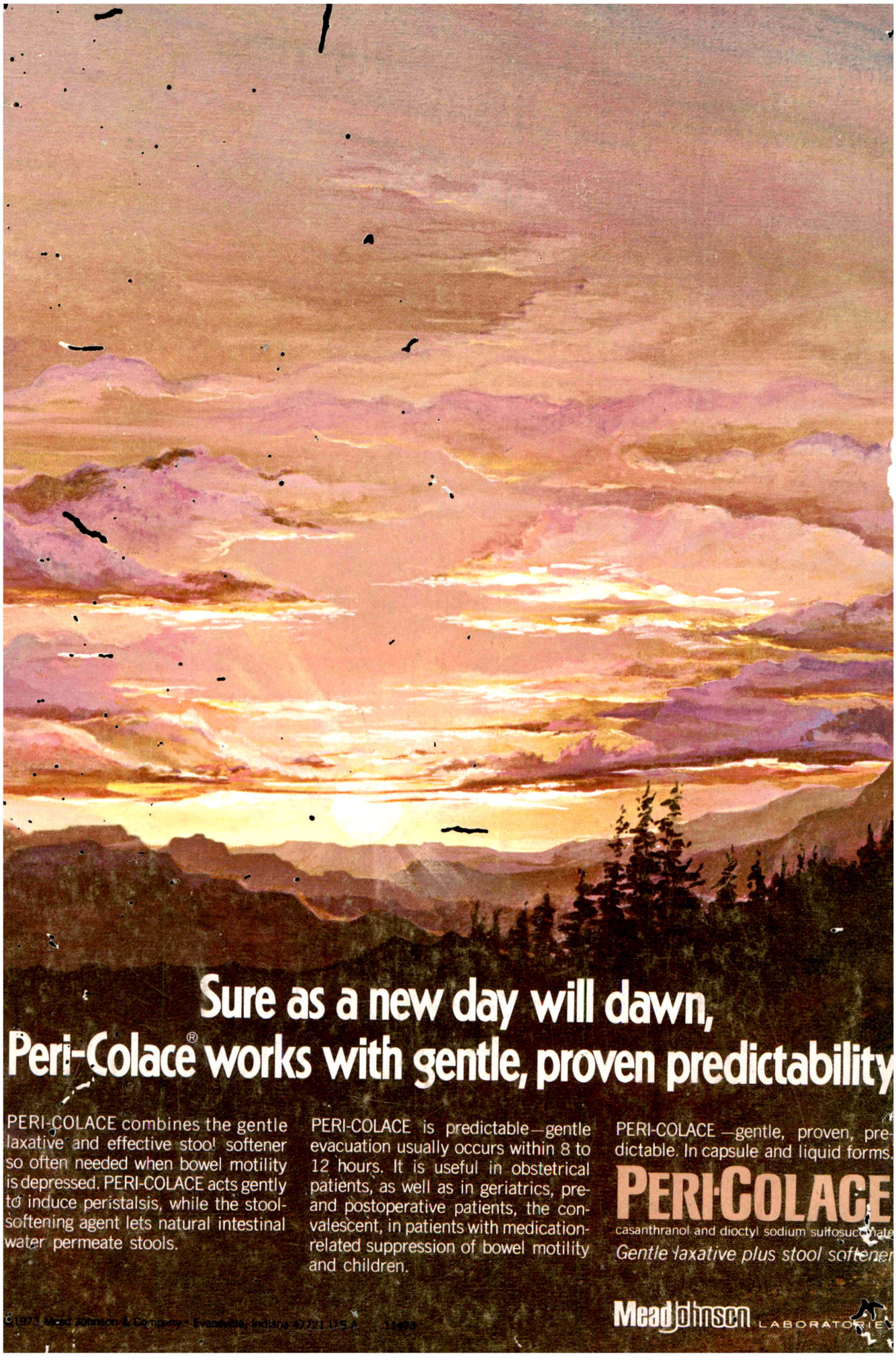
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